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EDITED BY
JOHN J. ABEL
The Johns Hopkins University
AND
ARTHUR R. CUSHNY
University of Edinburgh
IN ASSOCIATION WITH

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J. T. CASH,
University of Aberdeen
A. J. CLARK,
University of London
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University and Bellevue Hospital Medical
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ON THE INFLUENCE OF COLLOIDS ON THE ACTION OF NON-COLLOIDAL DRUGS

V. A FURTHER ANALYSIS OF THE AUGMENTOR EFFECT OF LECITHIN ON THE ACTION OF PILOCARPINE

W. STORM VAN LEEUWEN AND A. V. SZENT GYÖRGYI

From the Pharmacotherapeutical Institute of the University of Leiden

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Work done during the last few years has shown that the intensity of the action which a certain quantity of a drug exerts after injection into an animal, may be largely influenced by the presence in the blood (and elsewhere in the body of the animal) of substances which are able to inhibit or augment the action of the drug.

Influences of an inhibiting influence of colloids on the action of pilocarpine, atropine, cocaine have been given in former publications (1, 2, 3, 4, 5). In communication III of this series we have shown that the action of pilocarpine on the isolated gut can be increased very considerably by a number of colloids, and the importance of this phenomenon for physiological and pathological reactions was pointed out. The matter was further studied in other papers (6, 7, 8,) and has thus far disclosed the following facts.

The action of pilocarpine on the isolated intestine of the cat can be increased by the addition of small amounts of serum, lecithin, kephaline, cerebrin, cholesterin, peptone (Witte) (6, 8).

The action of histamine on the isolated intestine is increased by lecithin (8).

The action of adrenaline on the bloodpressure of the decapitated cat and (in some instances) on the bloodpressure of the

decerebrated rabbit can be augmented by peptone or by a dialysate of peptone (7).

Moreover it was shown that the blood of normal cats contains substances which intensify the action of adrenaline and in the case of one cat, particularly insensitive, its susceptibility to adrenaline was increased more than tenfold by the action of minute quantities of normal human serum or normal cat serum (7).

Another instance of the augmentor effect of colloidal substances has been given recently by Löffler and Spiro (9), who showed that ascites fluid (inactivated at 52°) increased the action of histamine and adrenaline on the isolated intestine.

The only instances of a similar intensifying action of colloids on the action of non-colloidal drugs that we found in the older literature were observations of de Waele (10) and of Koch (11). De Waele stated that small amounts of lecithin increased the action of alkaloids and of salts of alkaloids, whereas large doses of lecithin exerted an inhibiting influence. Koch, who made a very important study of the behavior of lipoids found that the action of strychnine given as the free base to a frog by subcutaneous injection was accelerated by the addition of lecithin; the free base acting as quickly in this case as otherwise the salts of strychnine do. Both authors ascribed the augmenting action of lecithin to the fact that alkaloids and their salts being readily soluble in lipoids can be more easily transported in the body and can be made to diffuse quicker into tissues and cells after being mixed with lipoid emulsions.

With regard to the possibility of subsequently finding an explanation for the augmentor action of colloids, a paper of Flexner and Noguchi (12) on the influence of colloids on the diffusion of haemolysins, published in 1906 is worth mentioning here. They found that the rate of diffusion of saponine into a gelatine layer was increased by the addition of agar.

The references given above all deal with the action of lipoids on non-colloidal drugs. The influence of lipoids on colloidal drugs has been studied much more extensively. It is not necessary to give a summary of all the work in this field. However,

with regard to researches to be reported in later communications, we want to call attention to two instances of an augmentor action of lecithin, i.e., the influence of lecithin on cobra hemolysin and the influence of lecithin on the hemolytic action of silicic acid.

The activating influence of lecithin on the hemolytic action of cobra toxin was first studied by Kyes (13). Since then a great number of papers have been published on the subject and various theories on this lecithin action have been proposed, the question seems to have been settled by Lüdecke (14) and Neuberg and Rosenberg (15), who showed that the hemolytic action of lecithin-cobratxin mixtures depends on the splitting of lecithin by cobra-toxin; this gives rise to the formation of monostearyl-lecithin or monopalmityl-lecithin, both of which substances have a hemolytic action.

The hemolytic effect of silicic acid-lecithin mixtures was discovered by Landsteiner and Jagic (16). Colloidal solutions of silicic acid cause agglutination of red bloodcells and these solutions have also a slight hemolytic effect. This hemolytic action can be greatly increased by the action of lecithin and Arrhenius (17) found afterwards that non-colloidal solutions of boric acids can (in higher concentrations) be made hemolytic by lecithin in an analogous way. Extensive references on the subject can be found in a communication of Landsteiner (18).

From the facts related above, it may be taken for granted, in our opinion that the intensifying action which colloidal and eventually non-colloidal substances may exert on the action of drugs and normal constituents of the body (adrenaline, histamine, choline) is of great physiological importance, and is a matter deserving further analysis.

Our communication III of the present series (8) dealt with the augmentor effect of lipoids on the action of pilocarpine and histamine on the isolated gut. As has been pointed out in that paper our work with the lipoids and especially our work with lecithin gave inconstant results. Very often the action of pilocarpine and histamine was largely increased by lecithin, but sometimes the lecithin for an unknown reason failed to act. Further researches however, which are described in the present communication, have cleared up this matter considerably.

In the greater number of the experiments with lecithin described in our former publications we used a sample of lecithin which contained a large amount of kephaline. We knew that pure kephaline has a very strong "augmentor" effect on pilocarpine action, and though this action (on account of a technical mistake, made at that time) was not entirely a constant one, it was much stronger and much more certain than that of the lecithin-kephaline mixture. It was therefore thought possible that the irregularity of the action of the lecithin sample might be explained by assuming that the augmenting effect of these samples depended on the kephaline content only, whereas the lecithin per se was inactive or even exerted an inhibiting influence.

The correctness of this assumption could be tested, for we had at our disposal a small amount of absolutely pure and kephaline-free lecithin, a sample of pure and lecithin-free kephaline and a mixture of known amounts of lecithin and kephaline. All these samples were obtained by the kindness of Dr. Levene from the Rockefeller Institute.

The first point to determine was, whether pure kephaline could be demonstrated to have with constancy an augmentor effect. In our former work kephaline had not always acted. We soon found however, that in those experiments we had sometimes worked with kephaline emulsions of too small a degree of dispersion. In the later experiments a certain amount of kephaline was first dissolved in ether and subsequently poured out in hot, slightly alkaline water, so that a 0.4 per cent emulsion resulted. This emulsion was boiled for three minutes and enough salt was added to render the solution isotonic. Quantities varying from 0.1 cc. to 0.5 cc. of this solution added to 75 cc. of Tyrode solution in which an isolated piece of gut was suspended, always gave positive results, i.e., they always had an augmentor effect on pilocarpine action.

The next step was to test the action of pure lecithin. It was shown that pure lecithin has practically no augmenting effect on pilocarpine action. Sometimes it seemed to inhibit the action of pilocarpine a little, sometimes it seemed to increase the pilocarpine action slightly; but on the whole it had no action at all.

After it had been shown, that pure kephaline has a very decided augmentor effect and pure lecithin has no such action at all, we investigated the behavior of our mixture of kephaline-lecithin. This mixture contains (according to the analysis made by Dr. Levene) 53.3 per cent of kephaline and 46.7 per cent of lecithin. Experiments with this mixture showed the same irregularities

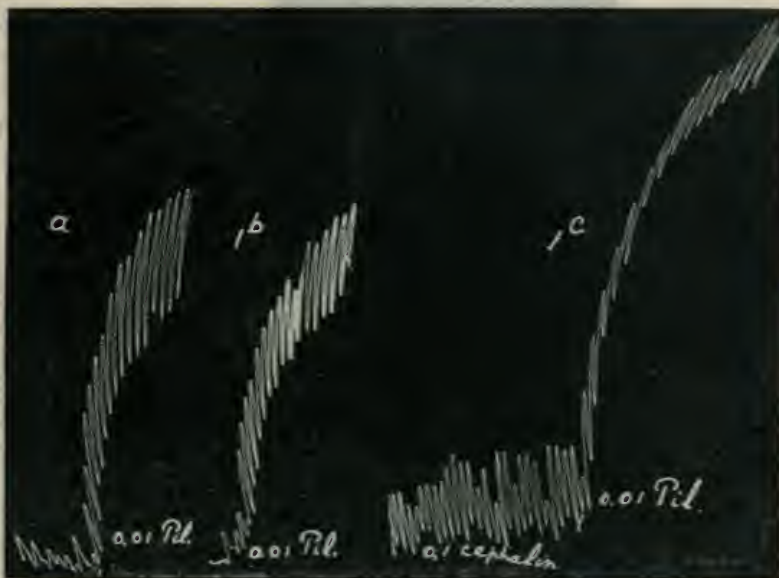


FIG. 1. AUGMENTOR ACTION OF KEPHALINE ON PILOCARPINE ACTION

Isolated intestine of the cat after removal of mucosa suspended in 75 cc. of Tyrode solution. *a*, Action of 0.01 mgm. of pilocarpine hydrochloride; *b*, *ibid.*; *c*, 0.1 cc. of 0.25 per cent emulsion of kephaline has been added to the Tyrode solution in which the loop of intestine is suspended; after five minutes 0.01 mgm. of pilocarpine hydrochloride is added.

as we had encountered with analogous preparations before; in some instances an intensifying action was present, in other instances it was absent.

Figure 1 gives an instance of the augmentor action of pure kephaline on pilocarpine and in figure 2 proves that pure lecithin is entirely negative in this respect.



FIG. 2. PURE LECITHIN ON PILOCARPINE ACTION

a and *b*, Action of 0.003 mgm. of pilocarpine hydrochloride; *c*, action of pilocarpine after 0.5 cc. of 0.25 per cent lecithin emulsion has been added; *d*, *ibid.* after 2.5 cc. of lecithin.

In figure 1, 0.01 mgm. of pilocarpine gives a strong contraction of the gut (fig. 1, (a and b). After 0.1 cc. of a kephaline emulsion has been added, the same dose of pilocarpine gives a stronger action (fig. 1, c).

In figure 2, a and b, 0.003 mgm. of pilocarpine is given. In figure 2, c and d the same dose of pilocarpine is given but five minutes before the addition of the pilocarpine $\frac{1}{2}$ respectively 2.5 cc. of a 0.1 per cent lecithin emulsion had been added to the gut.

After it had been found that kephaline exerts an augmenting effect on pilocarpine, whereas lecithin has no such action and after it had been found, that kephaline-lecithin mixtures gave irregular results, we were interested to study the influence of lecithin on the augmentor effect of kephaline. For this purpose we prepared the following solutions.

1. Kephaline was first dissolved in ether and this solution was poured out in water. The quantities of kephaline and water were so chosen that a 0.25 per cent emulsion of kephaline resulted.

2. Lecithin dissolved in ether, poured out in water, making a 0.25 per cent emulsion of lecithin.

3. Equal parts of 0.5 per cent of kephaline and 0.5 per cent of lecithin emulsions were mixed so that an emulsion containing 0.25 per cent of lecithin was obtained.

4. Equal quantities of kephaline and lecithin were dissolved in ether, the ether solutions mixed and then poured out in water. The quantities of each substance were so chosen that an emulsion containing 0.25 per cent of kephaline and 0.25 per cent of lecithin resulted.

The difference between solutions 3 and 4 consists in the fact, that in solution 3 lecithin and kephaline *emulsions* were mixed whereas in solution 4 lecithin and kephaline *solutions* were mixed and afterwards poured out in water; it was anticipated, that in solution 4 there would be a much closer contact between kephaline and lecithin than in solution 3.

The influence of the four solutions on pilocarpine action was studied. Solution 1 had the ordinary kephaline effect, solution 2 (pure lecithin) had no influence on pilocarpine action, solution

3 was active, but less active than solution 1, whereas solution 4 was inactive or only slightly active. In solutions 3 and 4 the kephaline content was equal to that in solution 1, so that in these experiments it has been proved, that the action of kephaline is inhibited by the addition of lecithin and this inhibitory effect is much more pronounced when kephaline and lecithin are first mixed as solution in ether, than when only emulsions of these lipoids in water are mixed.

In a former paper we stated that lecithin-kephaline mixtures do not only promote the action of pilocarpine, but also the action of histamine. We wanted to know whether in that case also the augmentor action depended on the kephaline content of the mixtures used. For that purpose we made a series of 8 experiments, in which the matter was investigated as carefully as possible. Since we were also interested to know how the lipoids used would influence the action of choline on the intestine we included this drug in this series.

These experiments were performed in the following way.

Three pieces of intestine from the same cat were suspended in Tyrode solution after removal of the mucosa. One piece was used for the pilocarpine experiments only; one piece for the histamine only and one piece for the choline only. In each case varying doses of the drug to be studied were given (and subsequently washed out again) till a dose was found which gave a contraction of moderate degree, and of constant intensity. It was assumed that a constant reaction was obtained when three subsequent applications of the same dose of the drug gave exactly the same results.

When a constant reaction was obtained the influence of the lipoid was studied. We used pure lecithin and pure kephaline, in 0.25 per cent emulsion. Since it appeared (as will be described in detail in a later publication) that ultrafiltrates of kephaline have the same augmentor effect as kephaline emulsions alone, we included the study of these ultrafiltrates in this series of experiments; in fact we often used the filtrate of kephaline alone, omitting the kephaline emulsion itself. The results of these experiments can be seen from the table on following page.

	TIMES STUDIED	AUGMENTOR EFFECT	NO AUGMENTOR EFFECT
Lecithine on pilocarpine.....	7	1*	6
Lecithine on histamine.....	6	5	1
Lecithine on choline.....	6		6
Kephaline on pilocarpine.....	8	8	
Kephaline on histamine.....	2	2	
Kephaline on choline.....	3	1	2
Ultrafiltrate of kephaline on pilocarpine.....	8	8	
Ultrafiltrate of kephaline on histamine.....	4	1†	3
Ultrafiltrate of kephaline on choline.....	5	3	2

* The lecithine emulsion used in this case had been kept in the laboratory in a small flask for three days.

† Very weak action.

This table shows the following facts.

1. The action of *pilocarpine* can always or nearly always be augmented by the addition of small amounts of kephaline or of an ultrafiltrate of kephaline emulsions, doses of 0.1 cc. of a 0.25 per cent solution being usually sufficient to produce the desired effect. Sometimes larger doses—0.5 cc. of the same emulsion—were necessary. The amount of active substances present in the ultrafiltrates can only have been very small; this point will be discussed in a later paper.

Pure lecithin has no influence on pilocarpine action. In one case there was decidedly an influence; the lecithin emulsion used in that particular experiment had been kept in a glass flask in the laboratory for three days, so the lecithin may have been deteriorated. The same emulsion was without any effect on pilocarpine action however the next day.

2. *Histamine* action is mostly augmented by the addition of pure lecithin. This augmentor effect was distinctly present in five cases and absent in one. However it was never so marked as the kephaline augmentor action on pilocarpine.

The influence of kephaline on histamine action was tested only twice, it was positive in both cases.

The most important point is, that the ultrafiltrate of kepha-

line, which was nearly always active on pilocarpine, was negative on histamine. In one case it seemed to exert a slight augmentor action.

3. *Choline* action was *not* augmented by lecithin, but sometimes by kerphalin and by the ultrafiltrate of kephaline.

The inconstancy of the kephaline effect on choline action may be ascribed to the fact that the concentration-action curve of

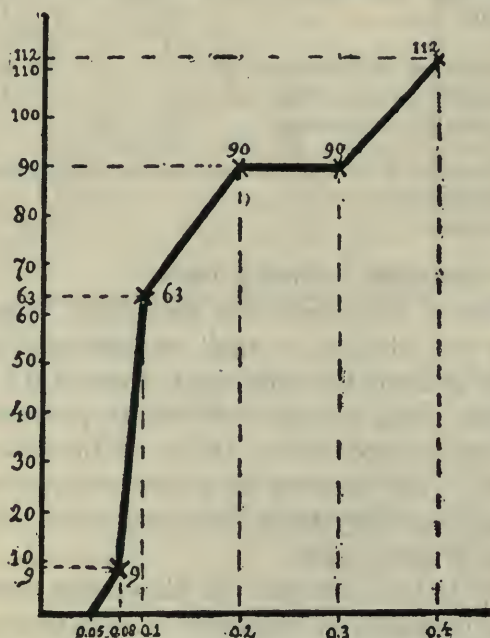


FIG. 3. CONCENTRATION-ACTION CURVE OF PILOCARPINE

this drug is not nearly so steep as that of pilocarpine, so that a small augmentor action, which in the case of pilocarpine may show a very distinct increase in the curve, may escape detection in the case of choline.

Figures 3, 4 and 5 give instances of concentration-action curves pilocarpine, histamine and choline, each curve has been taken on a separate piece of intestine, so that they do not offer absolutely comparable results, moreover the steepness of the curve is of course depended on the unit of magnitude chosen in plotting the ordinates and these units cannot be made equal in experiments with different drugs on different pieces of intestine.

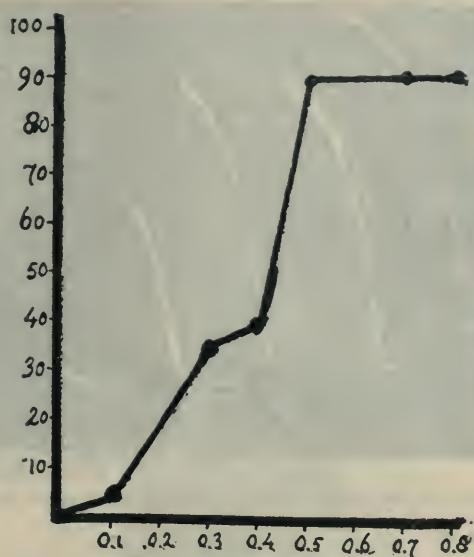


FIG. 4. CONCENTRATION-ACTION CURVE OF HISTAMINE



FIG. 5. CONCENTRATION-ACTION CURVE OF CHOLINE

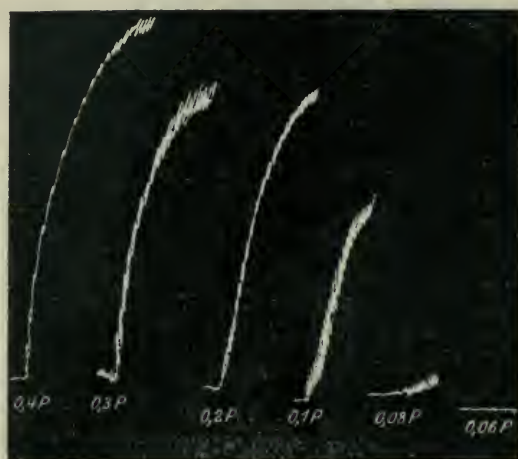


FIG. 6. ACTION OF VARIOUS DOSES OF PILOCARPINE ON ISOLATED GUT

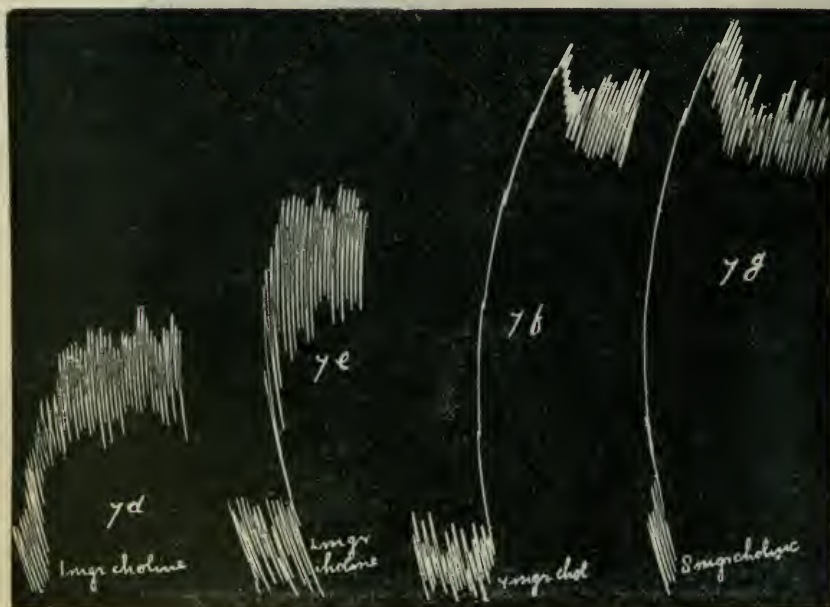


FIG. 7. ACTION OF VARIOUS DOSES OF CHOLINE ON THE ISOLATED GUT

The above illustration is incomplete. Sections *a*, *b*, and *c* were lost in the mail and could not be replaced.

Figures 6 and 7 give however the real shape of the concentration-action curves of pilocarpine and choline as they were taken in experiments which were according to our experience typical for these drugs. Moreover the fact that the pilocarpine and histamine curves are steeper than that of choline can be proved objectively by the following considerations.

In the pilocarpine curve the action of the drug (increase of tonus of the isolated intestine) is increased ten times when the dose of the drug (and thereby the concentration) is raised less than three times (from 0.08 to 0.2).

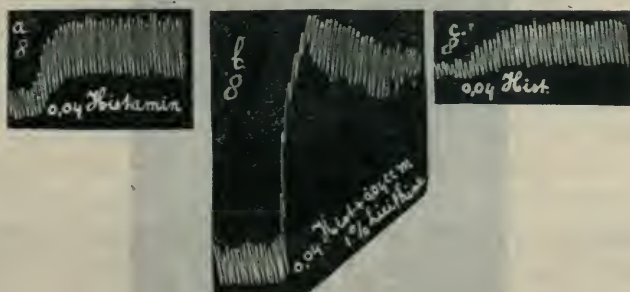


FIG. 8. AUGMENTOR INFLUENCE OF LECITHIN ON HISTAMINE ACTION

a and *c*, Action of 0.04 mgm. of histamine; *b*, action of 0.04 mgm. of histamine + 0.04 cc. of 1 per cent lecithin emulsion.

In the histamine curve the action is increased nearly twenty times by raising the concentration about five times.

In the choline curve the action is increased less than ten times when the concentration is raised from 0.2 to 4, i.e., twenty times.

The experiments related above then have partly cleared up the difficulties met with in our former researches on the augmentor effect of lipoids. On the other hand it had been shown that the question is a very complex one. Pilocarpine action can be augmented very distinctly by kephaline and not by lecithin, whereas histamine action is increased by pure lecithin and by kephaline (an instance of the augmenting action of pure lecithin on histamine is given in figure 8).

An ultrafiltrate of kephaline is very active on pilocarpine and inactive on histamine.

Choline action can not be augmented by lecithin. Kepheline and ultrafiltrates of kephaline are active, though inconstantly. The reason for this inconstancy has been pointed out above.

The fact that ultrafiltrates of kephaline are highly active on the action of pilocarpine, shows that some substances of non-colloidal nature may be responsible for part of the augmentor



FIG. 9. STIMULATING ACTION OF KEPHALINE

One cubic centimeter of 0.25 per cent of kephaline emulsion is added to the gut and after five minutes 0.05 mgm. histamine.

action of colloids. This observation may throw a new light on the biological function of lipoids, a matter which will be discussed in a later communication.

There is however another point which we want to bring into discussion now.

We have till now always spoken of an intensifying or augmentor effect of lipoids on drug action. The question arises whether the

expression is right. Often the lipoids studied will per se exert a stimulating influence on the isolated intestine so that there is a possibility that the effect of the lipoids is not an augmentor one, but that these lipoids only add their stimulating action to the stimulating action of pilocarpine. The whole matter would in that case be simply a case of pure synergic effect of two drugs.

It cannot be denied that kephaline emulsion may have a stimulating action on the isolated gut, as is seen from figure 9. We are of the opinion however, that it is quite certain that in addition to this stimulating action the lipoids have an augmentor action also. This assumption is proved by the following facts.

Often 0.1 cc. of a kephaline emulsion will have a very strong augmentor action on pilocarpine action, whereas 0.5 cc. of the kephaline emulsion alone is not able to stimulate the intestine in any considerable degree. Moreover often the augmentor effect will persist after the kephaline emulsion has been "washed out," so that only traces of kephaline can have stayed behind.

Finally if there was only a pure addition of kephaline and pilocarpine action it would be very difficult to understand, why kephaline ultrafiltrates increase the action of pilocarpine but do not increase the action of histamine.

Another possible explanation for the "augmentor" action of lipoids is to ascribe the effect of the lipoids merely to an increase of the general conditions of the isolated intestine analogous to the influence of lipoids on the hypodynamic heart in Clark's experiments (19). This assumption can also be proved to be incorrect by the fact that kephaline ultrafiltrates increase the action of pilocarpine and do not increase histamine action, whereas the reverse is true for lecithin.

We feel justified, then, in stating, that kephaline emulsions contain at least one substance which augments the pilocarpine and choline action on smooth musculature and one substance which stimulates the gut. The question whether those two substances are identical must remain open for the present time. Preliminary experiments in this direction have made it probable that they are not identical.

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ON RHODODENDRON POISONING

S. W. HARDIKAR

Pharmacological Laboratory, University of Edinburgh

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Some time ago some sheep in the neighborhood were reported to have shown symptoms of poisoning from eating rhododendron leaves, and my attention was drawn to the subject by Professor Cushny. Similar epidemic poisoning in sheep from eating rhododendron has been recorded previously by Parkinson (1) and Slipper (2). The chief symptoms are stated to be salivation, vomiting, weakness and staggering and death in a few instances. The poisonous constituent is stated by Plugge (3) to be andromedotoxin, which has also been found in a number of other Ericaceae. It was first prepared by Eykman (3) in 1882 from *Andromeda Japonica* and called by him, after the Japanese name of the plant "Asebotoxin." Plugge (5) who also found it about the same time, first in that plant and later in *A. polifolia* (6), *A. calyculata* (7), *A. catesbaei*, and in several other Ericaceae not belonging to the *Andromeda* genus, e.g., *Azalea Indica* and *Rhododendron Ponticum* (8), gave it the name of *Andromedotoxin*.

A systematic investigation of the substance, chemical and pharmacological, was made by H. de Zaayer in 1886, and a summary of his dissertation (9) (in Dutch) was published in 1887 by Plugge (3).

CHEMICAL

I isolated andromedotoxin from the rhododendron leaves by a method similar to that of de Zaayer. The leaves were cleaned, dried and powdered, and extracted with 90 per cent alcohol. Most of the alcohol was distilled off, the concentrated tincture further evaporated on a water bath, then taken up in water, precipitated with neutral lead acetate, filtered and the filtrate

rendered lead-free by H_2S . It was concentrated to a small volume on a water-bath, and shaken out repeatedly with ether and then with chloroform. According to Plugge, the poison should have been obtained in a crystalline form on evaporating the chloroform. But I found that the chloroform extracted almost nothing by mere shaking in a separating funnel; the active principle remained behind in the watery solution, which was then evaporated nearly to dryness, taken up in a small amount of 90 per cent alcohol and precipitated with an equal quantity of ether. This was repeated several times, till the alcohol-ether mixture extracted nothing more. The precipitate on being tested on a rat was found to be practically inert.

The alcohol-ether mixture was evaporated on a water bath and the residue taken up in distilled water in which it dissolved completely; it was neutralised with NaOH and was used in the larger number of experiments.

Later on, it was found that the poison could be obtained by extracting with chloroform in a Soxhlet apparatus, the concentrated watery solution—or rather its residue left on driving off as much as possible of the water—mixed with clean saw-dust (itself previously extracted with CHCl_3). The poison thus obtained could be purified by washing with ether or acetone in which it is insoluble. Messrs. Duncan, Flockhart and Company then handled for me a large amount of leaves, which were chopped up and extracted with boiling water, instead of alcohol, as was done by de Zaayer also, and prepared some pure andromedotoxin. This was used to confirm my earlier observations with less pure preparations and also for an examination of its chemical properties carried out under Professor Barger by Mrs. Stedman, to whom I am indebted for the following note on the chemical formula and molecular weight.

Pure andromedotoxin occurs in fine white crystalline needles containing no water of crystallisation. The best solvent for recrystallisation was found to be Butyl alcohol. The melting point is given by de Zaayer as $228-229^\circ$, but was found to be 258° with our preparation.

The molecular weight determined by Barger's microscopic

method was found to be 357; and by Beckmann's method (boiling point in methyl alcohol solution) 361.

Analysis for C and H. Specimen melted at 258°; it was dried at 110°.

I. 0.17955 grams gave 0.42355 grams CO₂ and 0.14205 H₂O; = C, 64.33 per cent; H, 8.79 per cent.

II. 0.1415 grams gave 0.33285 grams CO₂, and 0.1124 grams H₂O = C, 64.09 per cent; H, 8.82 per cent.

The formulae most nearly agreeing with these analytical results are: C₁₉H₃₂O₆ : C = 64.04 per cent; H = 8.98 per cent; M = 356; and C₁₉H₃₀O₆ : C = 64.41 per cent; H = 8.47 per cent; M = 354.

De Zaayer did not determine the molecular weight; his analysis gave C = 63.765 per cent, H = 8.750 per cent, and the formula assigned by him was C₃₁H₅₁O₁₀.

Andromedotoxin is soluble in water and alcohol and to a less extent in amyl alcohol, chloroform, ether and benzol; insoluble in petroleum ether. A point of importance is that it is much more soluble in cold than in boiling water, the relative solubility figures according to de Zaayer being 2.81 per cent at 12°C. and 0.87 per cent in boiling water.

He found that its solutions in water, alcohol and amyl alcohol are laevo-rotatory and in chloroform, dextro-rotatory. It belongs to the class of indifferent, nitrogen-free bodies; its solutions are neutral in reaction; it is not precipitated by the alkaloidal precipitants or metallic salts, e.g., Fe₂Cl₆, CuSO₄, HgCl₂, AgNO₃, AuCl₃, PtCl₄, neutral and basic lead acetate. It does not reduce Fehling's solution, and is not decomposed by dilute acetic acid at 100°C. On gentle heating with dilute H₂SO₄, HCl, or H₃PO₄, however, it gives a red color.

PHARMACOLOGICAL

De Zaayer found that the poison had no influence upon unicellular organisms like *Paramoecium coli* and *Opalina ranarum* even in a saturated solution. *Lumbricus terrestris* remained quite normal in a solution of 1:8000. In a strength of 1:400, the worms at the commencement made active movements, became

quiet shortly afterwards, but death did not occur until after the lapse of twenty-four hours. In frogs he observed vomiting, arrest of respiration, and a paralysis resembling that of curara. In mammals he describes the occurrence of emesis and purging, severe respiratory disturbance and dyspnoea, convulsions and death through arrest of respiration.

He ascribes the final paralysis to an action on the motor nerve ends, for the muscles respond to direct stimulation after they have ceased to do so to stimulation of the nerve. The emetic effect he thinks is due to a direct action upon the vomiting center, as vomiting occurred in a dog three minutes after a subcutaneous injection; the respiratory disturbance and arrest he attributes to a direct action upon the respiratory center rather than to a curara-like peripheral paralysis because they appear in the frog before any symptom of general paralysis; and in the rabbit the nerve ends are shown to be intact by the convulsions which attend the failure of respiration and which may be prevented by artificial inflation.

In rabbits the respiration is retarded without any previous acceleration; and the changes observed could not be due to an action on the vagus endings in the lungs, for section of the vagus does not produce similar changes. Throughout the experiment, the heart's action is normal.

Archangelsky (10) confirms the observations of de Zaayer and Plugge and adds that the direct excitability of muscle declines after the action on the motor nerve ends. The heart in the frog and mammals under andromedotoxin shows some resemblance to that in digitalis poisoning, but the systolic contraction is not so fully developed in the frog.

Hayashi and Muto (11) determined the excitability of the phrenics and other motor nerves, and of the muscles, to electric stimulation and came to the conclusion that andromedotoxin lessens the response of the motor nerves of the frog and rabbit so that they react quite normally to short stimulation, but fail when the stimulation continues for some time, and recover their excitability after rest. In non-fatal doses, the respiratory disturbance in the rabbit is due to a central action, for the phrenic

is not altered in excitability. The cause of death is arrest of respiration from paralysis of the phrenics; the respiratory center is still excitable, for if artificial respiration is suspended, the accessory muscles, especially the elevators of the ribs and the alae nasi, maintain a rhythmical movement for about thirty seconds.

It is difficult to reconcile these conflicting opinions as to the condition of the respiratory center and the cause of death. It seems unlikely that the laboured breathing which is one of the early symptoms can arise from a curara-like paralysis of the motor nerve ends; yet all observers agree that this is developed to some extent when the respiration fails. I therefore made a number of experiments to elucidate this question, and this developed into a full examination of the action.

The solutions actually used in most of the experiments were dark amber in color and probably contained some Rhododendrin and Rhododendrol, for after some weeks a crystalline deposit was found at the bottom which was soluble on warming. Rhododendrin is, according to Archangelsky, pharmacologically inactive; while Rhododendrol has an action similar to that of camphor on frogs, but even 0.19 gram causes no symptoms in rabbits or frogs. In my experiments the solutions were given in quantities of 0.06 cc. per kilo for rabbits, so that the effect of the rhododendrol may be disregarded. In addition the results were confirmed by experiments with pure andromedotoxin. The lethal doses by subcutaneous administration of the various preparations per kilo of the animal's body weight were 2.5 to 3 mgm. in the frog and 0.3 mgm. in the rabbit, of andromedotoxin.

SYMPTOMS

The following summary of an experiment will serve to illustrate the phenomena of poisoning:

May 2, 1921. Rabbit, female, weight 1 kilo: Laboratory temperature 14.5°C.

9.30 a.m. Respiration 30, heart 35, per ten seconds.

9.36 a.m. Injected 0.28 mgm. andromedotoxin subcutaneously into flank.

- 9.39 a.m. Respiration 24.
9.40 a.m. Respiration 20: uneasy.
9.43 a.m. Respiration 11, heart 33; biting movements. Got out of tray.
9.48 a.m. Respiration 6; laboured; mouth breathing; ears feel hot.
9.53 a.m. Tends to fall on the side, but recovers itself.
9.59 a.m. Heart 42; ran out of tray to a corner of the room.
10.00 a.m. Respiration 12; saliva dribbling from the mouth; head falls on its side, but tries occasionally to raise it.
10.05 a.m. Falls on side: pupils small and unequal in size, not responsive to light.
10.10 a.m. Respiration 10, heart 40.
10.26 a.m. Respiration 6; limbs give way when it tries to rise.
10.39 a.m. Respiration 4: heart 28.
11.03 a.m. Respiration 8: heart 28.
11.07 a.m. Convulsion.
11.10 a.m. Lying absolutely limp; no response on strongly pinching the skin; mouth breathing.
11.50 a.m. Respiration 11, heart 21
12.36 p.m. Convulsive tremor of limbs, mouth breathing; resp. 11 heart 19.
1.26 p.m. Respiration 12: heart 22.
2.35 p.m. Respiration 18: hear 19.
3.30 p.m. Respiration 12: heart 21.
4.20 p.m. Respiration 12: heart 19.
5.45 p.m. Respiration 12: heart 17.
9.30 p.m. Respiration 7: heart 13.
May 3, 1921
9.20 a.m. Found dead and in rigor.

In the rabbit within a few minutes after the subcutaneous injection of a fatal dose (0.28 mgm. per kilo) the frequency of respiration is greatly reduced; the movements become irregular in time and amplitude and assume Cheyne-Stokes character, sometimes long pauses of thirty seconds or more occurring and sometimes a series of movements following each other at a more rapid rate than before administration of the poison, to be followed by a pause. The respiratory movements are spasmodic, labored and noisy, the expiration in particular prolonged and accompanied by loud wheezing as in asthmatic breathing. The alae

nasi are seen to be working vigorously and mouth breathing generally occurs. The whole picture indicates that the animal's efforts are all concentrated upon securing a sufficiency of air. The animal is at first uneasy and excited; a peculiar drawing-in movement of the neck occurs from time to time, or general convulsions occur, and movements resembling those of vomiting also are seen. There is salivation, though this is not very marked in the rabbit, and repeated passage of urine and faeces. Muscular weakness (going on to paralysis) soon manifests itself; the animal generally retires to a corner of the cage or tray; if forced to move, its gait is awkward and uncertain and it often falls over on its side; it soon becomes unable to hold its head erect, all muscular tone is lost, the limbs give way under the body, and it lies in a helpless condition, apparently unaffected by its surroundings or by its unnatural posture. The respiration becomes progressively weaker until death takes place which is preceded by a convulsion. The heart is at first slowed for a few minutes, then beats much faster than before administration of the poison and after continuing at the increased rate for an hour or more, becomes progressively slower and weaker till death. It continues to beat for several minutes after the permanent arrest of respiration, and the auricles still continue to beat for a long time after the ventricles have stopped.

A dose of 1.4 mgm. per kilo hypodermically caused death in fifteen minutes; while 0.28 mgm. per kilo caused death in between twelve and twenty-four hours.

In the cat, the same series of symptoms is seen. There is much freer secretion of mucus from the nose; the saliva dribbles from the mouth; there is retching, and repeated and severe vomiting occurs—which is of course absent in the rabbit. This animal can stand much larger doses than the rabbit, however, recovery having occurred after 0.6 mgm. per kilo given subcutaneously, although the animal had not become quite steady on its legs even at the end of forty-four hours from the time of injection. The vomiting seems to be better elicited by smaller than by larger doses, which rapidly bring about the condition of narcosis and muscular weakness.

The frog, being able to remain alive in spite of a total stoppage of respiration, exhibits the phenomena of muscular paresis culminating in total paralysis much more clearly than the mammals, besides showing the effects observed in them. After the injection of 0.28 mgm. per kilo, the animal becomes uneasy for some time following the injection, but this soon passes away. The respiration at the end of four minutes is found to be much slowed and irregular, pauses of fifteen seconds or more occurring frequently. The mouth is widely opened, the animal often uttering a cry while doing so; vomiting movements occur repeatedly, the forelimbs being often carried to the mouth; it often remains still as if stupefied, with the mouth more or less open. Muscular weakness is shown in its not being able to hold the head erect and in fibrillation of the limb muscles; but with this dose, there is no complete arrest of respiration nor total general paralysis. Recovery follows in about one and one-fourth hours. As the dose is increased, the respiratory disturbance occurs earlier, is more severe and prolonged and the other symptoms are correspondingly severe. But it is not until six times this dose is given that approach to a complete muscular paralysis lasting for some time is seen, appearing about one hour after administration of the dose, while a certain amount of recovery is noticeable at the end of three hours.

While this muscular paralysis is developing, the vomiting is beginning to pass off and the animal remains still if left undisturbed, but if the toes are pinched, it jumps forward actively but its hind limbs trail behind it and the muscles remain fibrillating for a considerable time after the jump; also, the performance is not repeated if a stimulus is applied immediately after the jump, though, after a few minutes' rest it may jump almost as vigorously as before. As time goes on, the muscular tone is lost in an increasing degree, the limbs give way, the head cannot be held erect and the animal sinks into a heap. If stimulated at this stage, it may strike out its limbs vigorously though it is no longer capable of locomotion. Later on the response consists only in a feeble movement of the limbs and finally even this disappears. If laid on its back in the earliest stages it soon recovers

itself, but as time goes on it remains for a considerable period before attempting to regain the normal position, the efforts become ineffective and finally the animal can be laid in any position without evoking any movement on its part.

As already pointed out, the respiratory throat movements are almost the last to disappear and precede or accompany attempts at movement, whether of the whole body or of the limbs alone. As regards the circulation, the rate of the heart may be accelerated during the stage of excitement and vomiting, but later on there is progressive slowing and weakening of the force of contraction. The absence of circulation in the web as an index of death is apt to be misleading, for I noticed more than once that the capillaries there were empty and the larger veins along the toes engorged and the blood showing no movement, while the heart beats could still be seen through the body wall.

RESPIRATION

The changes in the breathing have already been described as a striking feature in the general action. When the movements are recorded for anaesthetised or decerebrate animals, the changes in the rate of respiration are very marked, as is seen in figure 1. When the vagi are divided (fig. 1, *c*) the breathing immediately became faster; it did not reach the original rate, but the slowing did not seem greater than that seen in unpoisoned animals in which the vagi had been divided.

This result was confirmed repeatedly and suggested that the marked primary slowing of the respiration is not a central action, but arises from some action in the periphery which results in inhibitory impulses being carried to the center along the afferent vagus fibres. This was confirmed by experiments in which the vagi were divided before the injection of andromedotoxin was made and in which no such primary slowing occurred from it. Similarly when the primary slowing from the poison had been reduced by division of the vagi, (fig. 1, *d, e*) a new injection caused no repetition of the change; the inhalation of CO_2 now acted in the same way as in unpoisoned animals after division of the vagi, that is, by increasing the depth of the movements without quickening

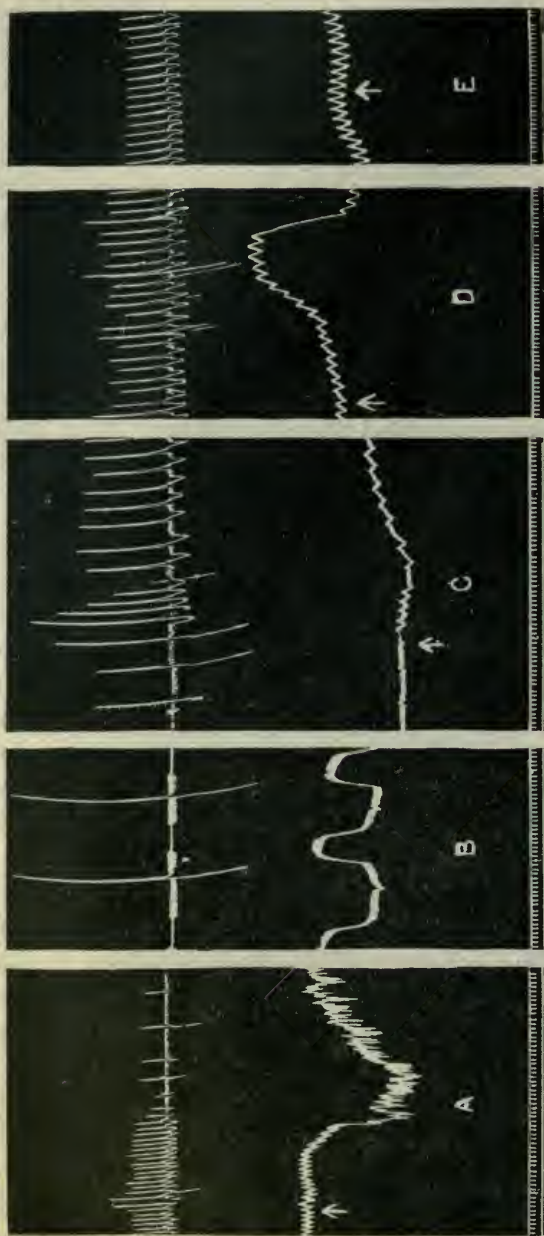


FIG. 1. CAT, 2000 GRAMS; PARALDEHYDE ANESTHESIA; TRACHEOTOMY

Respiration record from tambour connected with a T-tube in the trachea; downstroke = inspiration; carotid blood pressure. Time in 2 seconds. Injections into the jugular vein. *a*, shows the first injection, 0.2 mgm.; *b*, shows the condition five minutes after injection; *c*, 20 minutes after injection; *d*, second injection, 0.2 mgm.; *e*, thirty-eight minutes after the first injection; *e*, third injection, 0.2 mgm., thirty-eight minutes after the first injection.

the rate, so that the respiratory center does not seem to be significantly depressed at this stage.

This stimulation of the peripheral mechanism of the inhibitory vagus fibres to the respiratory center is not the cause of the final arrest of the breathing. Here my findings are in agreement with those of Hayashi and Muto that the phrenics are almost totally paralyzed by doses which cause a complete arrest of respiration: they may sometimes respond to the stimulus once or twice, but are then found to be completely paralyzed.

But I also found, contrary to their observation, that the diaphragm showed the same condition on direct stimulation; it either did not respond at all, or failed to do so after repeating the stimulus once or twice.

The other motor nerves, e.g., the sciatic and femoral, and the skeletal muscles, e.g., those of the calf, the glutei and intercostals, were at this time in a much better condition, as seen by response to stimulation directly applied or through the nerve. But I did not investigate their condition so fully as Hayashi and Muto, and can only state that the phrenics and diaphragm are affected earlier and more severely than the other motor nerves and the skeletal muscles supplied by them.

ACTION UPON THE BRONCHIAL MUSCLE

This was investigated by perfusing the lungs of a recently killed rabbit, through the pulmonary artery with a solution of the poison in gum-Ringer, and recording the tracheal pressure by means of a tambour the tube leading from which was tied into the trachea. Figure 2 shows the sudden rise of intratracheal pressure, caused by a solution of 1:1,000,000 of the poison. The rise is followed by a slow fall; this may have been due to a bursting of some alveoli during the rise and escape of air out of them, as air was found thus to escape on trying to reinflate the lungs. The presence of atropine, 1:100,000, in the perfusing fluid prevented this action even when the strength of the poison was increased four times, i.e., 1:250,000; though it could be obtained on washing off the atropinised solution with gum-Ringer and reperfusing with the poison alone. This rise can only be due to a

vigorous contraction of the bronchial muscle, through the vagus. This direct evidence, together with the asthmatic type of dyspnoea seen under the poison, leaves no doubt that it causes at first a strong constriction of the bronchial muscle.

The development in the perfused lung shows that this is of peripheral origin, while its absence after atropine eliminates any

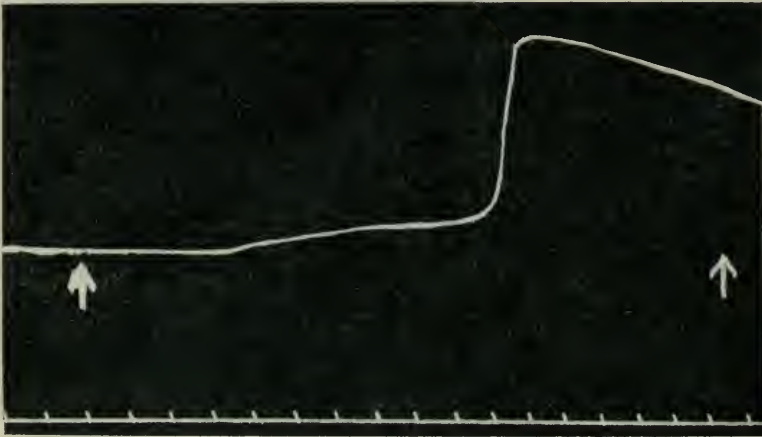


FIG. 2. PERFUSION OF THE LUNGS OF RABBIT WITH 1: 1,000,000 ANDROMEDOTOXIN IN GUM-RINGER

The upper line registers intratracheal pressure; the lower, the outflow; each interval is 3.5 cc. At the arrow marks, the poison was turned on and off.

direct action on the muscle. The bronchial constriction thus arises from stimulation of the vagus terminations in the bronchial muscle.

This bronchial action is independent of the changes in rhythm of the respiration which has been described above, for the latter is unchanged after doses of atropine which prevent any bronchial constriction.

ACTION ON THE BRONCHIAL MUCOUS GLANDS

With the doses which produce respiratory embarrassment the breathing is accompanied with moist gurgling sounds, and frothy mucus may also appear at the nostrils, showing that the secretion

is increased. Post mortem, the trachea is also found to contain frothy mucus. This secretion is absent when atropine has been injected previously, and also under an anaesthetic, so that it seems to be brought about by central nervous changes and not by direct action on the glands.

HEART

Within a short time after the intravenous administration of a large dose, e.g., 0.45 to 0.60 mgm. of toxin per kilo, to a rabbit, there is a considerable initial fall of blood pressure and slowing of the heart to almost half its previous rate. There is then a slight temporary rise followed by a continuous fall very nearly to zero. After the final arrest of respiration, the heart continues to beat for several minutes, though at a much slower rate. Death from these doses occurred in two to six minutes. In doses which are not so rapidly fatal (0.15 to 0.30 mgm. per kilo) the blood pressure after the preliminary fall attains very nearly its previous level and may go even above it during the respiratory pauses, and according to the magnitude of the dose, shows a tendency either to fall slowly and steadily or to remain fairly steady, the fall being no more than might be expected in an animal kept for hours under anesthesia.

The rate of the heart during the primary fall of blood pressure is much slowed or it may be arrested temporarily; it then rises again and often exceeds that previous to the administration of the poison; this is followed by some variations in the rate and on the whole a tendency towards a steady slowing. The following tabulated summary of an experiment will bring out these points.

After the heart rate and blood pressure have recovered from a sublethal injection, a second small dose has not the marked effect on them of the first and in fact may have no significant action (fig. 1, *d*), but a large dose reduces the blood pressure rapidly to nearly zero, and although on inspection the heart is found to continue to beat, the action is not strong enough to maintain pulsations on the blood pressure tracing.

The early slowing with fall of blood pressure arises from inhibitory action, for it is absent after atropine, though it is not

prevented by previous division of the vāgi. Accordingly the strength of stimulus applied to the vagus required to produce

Experiment 41. Rabbit, 1500 grams. Urethane anesthesia, natural respiration

TIME FROM COMMENCE- MENT OF INJECTION	RESPIRATION		REMARKS	CIRCULATION		REMARKS	NOTES
	Rate per 10 seconds	Depth		Carotid blood pres- sure	Heart rate per 10 seconds		
	7	<i>mm.</i> 4		114	40		First injection 0.45 mgm. intra- venously
1' 0"	1	22	Pause of 10 seconds fol- lowed	118	27		
1'20"				58			
2'15"	5	45		140	22?		
2'45"	4	2		98	40		
3'15"	4	1		108	34		
3'45"	4	1		122	32?		
4'45"	5	0	Just percep- tible	156	40		Incomplete diastole in alternate beats
10' 0"	2	2, 5		136	40		
20' 0"	3	2		140	35		
40' 0"	3	3-4		118	31		
1.44'	3	2		84	25		
2.40'	3	3		54	20		
3.20'	3	2-3	48	21			
4.23'	3	2	46	19			

Second injection of 0.67 mgm.

20"	3	2-3		50	18		
1' 0"	3	2		38	20		
2' 0"			Not regis- tered	36	20		
13' 0"	Respiration stopped entirely. The heart was still beating.						

an arrest or slowing of the heart and a fall of blood pressure first decreases and then increases until stimulation of the vagus fails

altogether to produce any effect; in more than one experiment, instead of a fall, stimulation of the divided vagus caused a rise of blood pressure at this late stage without any change in the rate or amplitude of the cardiac beat. The vagus is thus first stimulated and then paralyzed. The increase in the rate of the heart in the rabbit amounted to about seven beats per ten seconds; this is not surprising seeing that normally there is very little vagus tone present in this animal. But both the primary slowing and the subsequent acceleration of the heart rate are well illustrated in an experiment of Archangelsky upon a dog, in which, 0.1 mgm. andromedotoxin was given intravenously. The rate before administration was 128 per minute; three minutes after injection it had fallen to 96, and in 22 minutes it had risen to 224. Here we have an acceleration of the heart to nearly double the previous rate. These observations showed that the poison first stimulates and then paralyzes the vagus mechanism in the heart.

The next step was to determine exactly which point of the vagus path was affected. For this purpose a rabbit, 2000 grams, was anaesthetised with urethane and artificial respiration maintained to eliminate any changes in the heart's action produced by imperfect aeration of blood as a result of the respiratory effects of the poison. A dose of 0.28 mgm. per kilo was given intravenously and when stimulation of the vagus ceased to have any effect upon the heart, the chest was opened and the heart observed directly, and was found beating at 26 per ten seconds. Nicotine was now injected (0.5 mgm.) to elicit the stimulant effect upon the vagus ganglia. It produced no effect. Pilocarpine was then injected in repeated doses of 1 mgm.; when 3 mgm. had been injected, the heart rate fell to 16 per ten seconds. Atropine was then injected slowly; when about 0.3 mgm. had been introduced, the rate rose to 26 per ten seconds again.

All these effects are however, better worked out in the frog heart which only differs from the mammalian, in that the ventricle is arrested in diastole instead of systole.

Figure 3, which is a tracing from a frog heart in situ, to which the poison was applied by dropping on it a solution of 1:40,000

shows that, whereas before the application of the poison a stimulation of the sino-auricular junction with the secondary coil at 45 mm. from the primary, was required to produce arrest of the heart, after the application, it was obtained with the coil at 60 and later on no arrest or even slowing was obtained even with the coil at 0. The activity of the vagus as shown by the effect of stimulation upon the heart, was restored by the application of pilocarpine, for an inhibition—though not total arrest—was then obtained with the coil at 40. This tracing thus brings out clearly

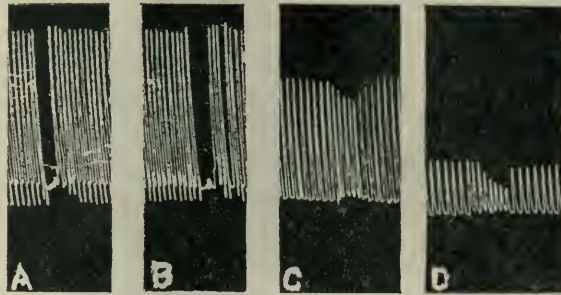


FIG. 3. TRACING OF FROG HEART IN SITU

Andromedotoxin, 1:40,000 dropped on; stimulation of sino-auricular junction; figures indicate distance of secondary from primary coil in millimeters. *a*, Before application of poison, stimulation at forty-five millimeters; *b*, Five minutes after *a* and soon after applying some drops of poison, stimulation at sixty millimeters; *c*, Eighteen minutes after *a*, stimulation at 0; *d*, Four minutes after *c* and after the application of pilocarpine, stimulation at 40.

the preliminary stimulation followed by paralysis of the post-ganglionic terminations of the vagus in the heart.

Besides these effects upon the heart attributable to the nerve mechanism there are also changes which are due to a direct action upon the heart for they occur after atropine. In the mammalian heart recorded directly in situ the beats are found to vary in their amplitude, alternate large and small beats occurring. In the perfused mammalian heart diastolic relaxation may at first be increased as also the range of contraction; very soon, however, both diastole and systole become less and at last the ventricles stop in partial systole, the auricles continuing to beat for a

considerable time. The most remarkable feature, however, is cardiac arrhythmia, the auricles and ventricles beating at different rates, as was observed in the heart in situ as well as in the isolated heart perfused with oxygenated Ringer's solution containing 1:3,000,000 andromedotoxin and 1:100,000 atropine (see

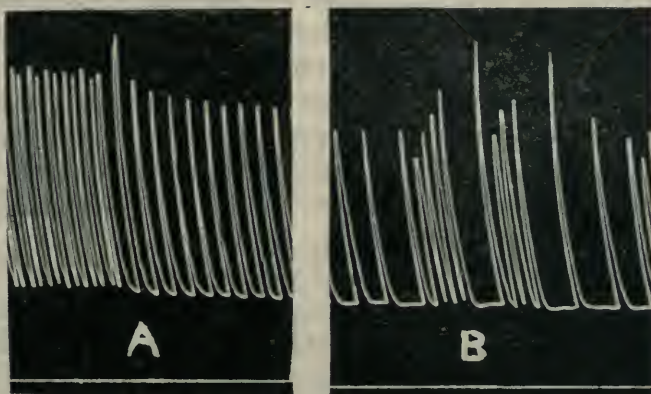


FIG. 4. PERFUSION OF ISOLATED RABBIT'S HEART

With 1:3,000,000 andromedotoxin and 1:100,000 atropine in Ringer's solution. *a*, Soon after the solution was turned on; *b*, Five minutes after *a*.



FIG. 5. PERFUSION OF FROG HEART IN SITU

With 1:425,000 andromedotoxin in frog-Ringer. *a*, Before application of poison; *b*, Thirty minutes after the poison was turned on; *c*, nearly two hours after *a*; twenty-five minutes after *c* Ringer was turned on; *d*, half an hour after Ringer was turned on.

fig. 4). The relative rates of auricle-ventricle became in a few minutes as 2:1 and then 3:1; later on, however, the ventricles began to beat faster and the ratio became 1:2.

Perfusion of the frog heart in situ through the vena cava with a solution of 1:1,000,000 induces partial a-v block, the rhythm becoming 2:1, and reduces the strength of the beat with slow, prolonged relaxation. A strength of 1:500,000 brings about the

same results in more marked form and the ventricle often remains in diastole for forty-five seconds or more. Recovery follows on changing the solution to Ringer. A solution of 1:400,000 (see fig. 5) in a few minutes slowed the rate of the ventricle to half the previous one, the rhythm becoming 2:1; and in less than eighteen minutes caused a diastolic arrest for forty seconds. The ventricle then began to beat again spontaneously and continued for 15 or 20 beats when another pause followed. These pauses increased in length and the number of beats in a cycle diminished, but total arrest did not follow upon perfusion for over an hour. During the long ventricular pauses, weak contractions of the auricles occurred; these gradually increased in magnitude until they were ultimately followed by the ventricular beat. Ringer solution restored the regularity of the rhythm, but at the end of nearly half an hour the rate still remained half the previous one. In other experiments a considerable reduction in the amplitude of the beat was seen. In a strength of 1:100,000, ventricular pauses of more than two and a half minutes' duration were brought about in less than fifteen minutes. The beats of the two auricles were also found not to be synchronous, and complete arrest of the ventricle occurred in less than fifty-five minutes. Merely turning on Ringer did not bring about spontaneous recovery, but it did follow when the heart was emptied by mechanical squeezing. In the frog, the ventricle was never found beating at a higher rate than the auricles, as was more than once observed in the mammal.

These experiments show that the direct action of the poison lies in diminishing the activity of the conducting tissue between the auricle and ventricle, thus producing a condition of heart block; also perhaps decreasing the excitability and increasing the fatiguability of the ventricle.

ACTION ON BLOOD VESSELS

Perfusion of the vessels of the frog by a cannula introduced into the left aorta, the fluid escaping through the cut sinus, gave uncertain results in concentrations up to about 1:100,000, but with solutions of 1:40,000 a marked fall in the outflow was ob-

served followed by an increase of over 50 per cent above the original amount, apparently from total loss of tone of the vessel walls.

The vascular effects in the mammal were investigated on the anaesthetised cat by plethysmographic records of the intestine, but no constant and definite results were obtained. Perfusion of the isolated rabbit's kidney indicated that 1:1,000,000 of andromedotoxin has no vaso-constrictor action; 1:500,000 has slight action, while 1:250,000 has a very strong constrictor effect, the flow with Ringer and with the poison being as 28:9.

EMETIC ACTION

As repeated vomiting is one of the very marked symptoms in andromedotoxin poisoning in cats and dogs and in the frog, it is of considerable interest to determine the seat of this action.

The following table gives the summary of results of the experiments performed on a cat without anaesthesia, with a view to determine the relative effective dose by the mouth and hypodermically. The same animal was used in all the experiments, which were performed at about the same time of day, and in the case of the oral administration, no food or drink was given on that day until just before the experiment, when a measured quantity of milk was offered; the poison dissolved in 5 cc. of milk was then given by the stomach tube, which was washed with 5 cc. more of milk.

These experiments give a ratio of hypodermic to oral dose as 3:4, but this is very different from the ratio in a purely centrally acting drug like apomorphine, where it is 1:28.5; while the times are ten minutes and twenty minutes respectively; they thus give no conclusive evidence in favour of central or peripheral action exclusively.

Another experiment of a different kind was therefore tried to solve this question. A cat, weight 2650 grams was decerebrated by Sherrington's method, the abdomen opened, the stomach exposed and incised and a wide glass tube with a flange at each end was introduced and the stomach wall sutured tightly around it; the oesophagus was divided just below the diaphragm com-

pletely between two ligatures, thus dividing the vagi. The other end of the tube was brought out of the abdominal wound and the wound sutured. A dose of 0.188 mgm. of toxin (i.e., 0.07 mgm. per kilo) which from another experiment on a decerebrated animal was known to be effective, was introduced into the jugular vein. The animal was then placed in the semidorsal position advised by Eggleston and Hatcher. Movements like those of vomiting were observed first after sixteen minutes and these were repeated several times. But there was no forcible expulsion of the stomach contents, and fluid introduced into the stomach trickled

Experiment 77. Cat, male, weight 3600 grams

DATE	DOSE	MODE OF ADMINISTRATION	RESULT
14/6/21	0.094 mgm. = 0.026 mgm. per kilo	Subcutaneous	No effect
15/6/21	0.141 mgm. = 0.039 mgm. per kilo	Subcutaneous	Vomiting in 10 minutes repeated 4 times in 37 minutes
17/6/21	0.094 mgm. = 0.026 mgm. per kilo	By the mouth	No effect
18/6/21	0.141 mgm. = 0.039 mgm. per kilo	By the mouth	No effect
21/6/2	0.188 mgm. = 0.52 mgm. per kilo	By the mouth	Vomiting (only once) in 20 minutes But there was very little constitutional effect produced and the animal readily took milk offered to it in 37 minutes

out slowly. As similar movements are, however, observed in rabbits and rats, which do not vomit, and may possibly be due to the respiratory distress and asphyxia, this experiment also must be looked upon as giving no conclusive evidence of any kind.

In frogs vomiting movements are seen after the injection of 0.28 to 1.13 mgm. per kilo but with 1.39 mgm. per kilo and above no vomiting movements are observed and the symptoms of general muscular paralysis come on rapidly. The greater emetic efficiency of smaller doses than of larger is sometimes regarded as a point in favour of the action being central: there does not seem

to be much weight in this however, for if a drug stimulates the vagus endings in a smaller concentration (or as the first part of its action) while in a stronger solution (or later in its action) paralyses them, it will behave in the same way. The emetic action can hardly be due to a local irritant effect for even a solution of 1:1000 dropped into the rabbit's eye, did not show any such action. It may be mentioned, however, that the pure substance applied to the skin of the face—but not, for example, to the back of the hands—causes a sensation of smarting. We must therefore leave this question unsolved for the present, only adding that if andromedotoxin should be found to produce emesis by peripheral action, this would be in line with what we have seen hitherto of its action on other functions.

ACTION UPON THE NERVOUS SYSTEM

One of the symptoms in sheep poisoned by *Rhododendron ponticum* was rolling and staggering about, as if drunk. The same symptoms are also seen in other animals. They are at first unsteady on their legs, sway about in walking and even when at rest and tend to fall over on one side; the hindquarters seem to be particularly weak; if the cage is tilted, the animal at first makes movements to keep its balance, but as time goes on it makes no attempt to recover its balance and appears to be in deep sleep and is not startled by a noise. A frog if placed on its back, does not attempt to recover its position, and sinks if placed in water. At first the animal draws away its legs if pinched or pressed, but later on this vanishes. The narcosis in a rabbit is deep enough to perform tracheotomy without any struggle on part of the animal. During recovery, consciousness and intelligence are regained early but swaying continues much longer; though when the eyes are bandaged and the animal is stood on its legs it does not fall. These effects were obtained in a cat with a subcutaneous dose of 0.6 mgm. per kilo from which recovery occurred but the animal was not quite steady on its legs and swayed in walking still at the end of forty-four hours. Muscular weakness of peripheral origin has obviously a very large share in this, but the narcotic effect is probably due to a direct action upon

the higher centers. The respiratory center, as we have seen, remains active till nearly the end. No specific disturbance of the spinal cord is noticed.

Experiments on reflex time in the frog with a chemical stimulus—3 per cent acetic acid—one hind limb being protected by ligation of the iliac artery, showed that the spinal cord is not affected by nearly one half the fatal dose, an effective response from the protected leg being obtained in almost the same time as before, while there was a great delay on the poisoned side. Here it is worth mentioning that even on the poisoned side it was the effective response (withdrawal of the foot from acid) that was delayed, for slight movements of the toes occurred in almost the same time as before. The delay would therefore seem to be due to a block on the motor path through the curare action of the poison, described below. In twenty-four hours after the injection, there was a recovery and effective response in very little longer time than before the poisoning. It may also be mentioned here that responsiveness to mechanical stimulation, e.g., a pinch, was retained longer than to the chemical.

NERVES AND STRIPED MUSCLE

The action on these in the frog was investigated by ligaturing one hind limb, the ligature passing beneath the sciatic nerve, and injecting the poison into the ligatured limb; also by two nerve muscle preparations, the nerve of one and the muscle of the other dipping in a solution of the poison and another experiment in which one muscle was immersed in poison and a control in frog-Ringer, and the response to electrical stimulation by faradic current tested. The following is a summary of the observations.

Experiment 68; June 7, 1921. Frog, weight 13 grams. Brain pithed. Right sciatic nerve exposed and the limb tied beneath it. Stimulation with faradic current—tongue feels current with coil at 150. Stimulation of tied limb: at 130 frog moves away. Stimulation of other limb: at 160 frog moves away.

11.52 Injected 0.00564 mgm. andromedotoxin in frog Ringer, under skin of the leg and foot of tied limb. There was a slight escape of fluid.

- 11.59. At 130, moves away, but movement is very imperfect in injected limb.
- 12.14 Moves away at 130, the injected limb being merely dragged along; on application of stimulus there is no contraction of leg or foot muscles, but on stimulation of the sciatic nerve the knee is extended: movement of muscles of the thigh.
- 1.30 At 130 moves away sometimes but not invariably.
- 2.10 No movement at 130, moves away at 80: uninjured leg at 180.
- 5.30 Does not move with stimulation of injected leg even with coil at 0: no response in muscles to direct stimulation. Stimulation of sciatic nerve effective (animal moves away) at 160 or even weaker current: unpoisoned leg, 180.

The poison is thus seen to produce paralysis of the motor nerve end and muscle before sensory action is manifested.

Experiment 53. On nerve muscle preparations

TIME	DISTANCE OF COIL IN MILLIGRAMS			
	No. 1. Nerve in poison (strength of poison 1:425,000)		No. 2. Muscle in poison	
	Stimulation of nerve	Stimulation of muscle	Stimulation of nerve	Stimulation of muscle
	250	225	235	160
40'	250	180	235 Muscle fibrillating	160
50'	250	180	210 Muscle fibrillating for long after stimulation	160
1 hr., 30'	250	175	235	160
Strength of solution increased to 1:200,000				
5'	250	175	235	160
15'	250	165	235 Feeble response	160
2 hrs., 15'	250	160	No response	130

Thus the nerve trunk was unaffected by a solution of 1:200,000 after two and a quarter hours.

Muscle immersed in 1:425,000 after forty minutes, began to show fibrillation for a considerable time after stimulation, but responded to the same strength of stimulus both through the nerve and direct, as before treatment, for one hour, thirty min-

utes. With a solution of 1:200,000 the response to stimulation through the nerve became feeble in fifteen minutes, and disappeared later. Direct stimulation was effective after two hours.

Experiment 64. On nerve muscle preparations

TIME	NO. 1. MUSCLE IN RINGER		NO. 2. MUSCLE IN POISON	
	Nerve	Muscle	Nerve	Muscle
3/6/21				
11.0	290	130	400	190 Tested in Ringer
11.2			Poison introduced 1:100,000	
11.15			330	140 Fibrillation long after contraction
11.55	300	150	320	150
12.40	300	150	None even at 0	130
1.40	260	150	None	90 Weak response
3.45	400	150	None	60 Weak response
4.30	400	150	None	None even at zero
4.55	340	140	None	None even at zero
5.0			Well washed in Ringer and immersed in Ringer	
4/6/21				
9.0	400	150	No response	

Thus a solution of 1:100,000 produced on immersion of muscle in it in:

13 minutes, Fibrillation on contraction to stimulus

1 hour, 38 minutes: Total loss of response to indirect stimulation

5 hours, 28 minutes: Total loss of excitability even to direct stimulation.

There was no recovery from this on substituting Ringer for the poison. The control preparation retained its excitability unimpaired at the end of 22 hours.

Thus the poison first paralyses the motor nerve ends but if the strength is sufficiently great, it also affects the muscle substance directly. Hayashi and Muto observed the increased fatiguability caused by the poison in the nerve ends in mammals and I have stated that after fatal doses the diaphragm fails to respond to stimulation of the phrenic nerve and sometimes also to direct stimulation.

INVOLUNTARY MUSCLE

Iris. When the poison is injected hypodermically or intravenously changes in the pupil are often observed; in rabbits it is extremely contracted at first and dilates later; in cats, the constriction was not noted. A solution of the pure toxin dropped into the eye did not produce any noticeable action on the pupil. It seems probable therefore that the effects from systemic administration are central in origin, and may be in part secondary to asphyxia.

Bronchial muscle. We have already mentioned in the rabbit the strong contraction of this brought about by a solution of 1:1,000,000.

Intestine. Pieces of isolated small intestine from the rabbit suspended in a solution of the poison in oxygenated Ringer's solution were used and their contractions recorded. A solution of 1:1,500,000 causes an increase of tone and amplitude of movement, the rate not being affected; even a solution of 1:2,000,000 produces a noticeable effect. Atropine 1:50,000 brought down the tone to normal, as also the range of contraction. Adrenaline continued to inhibit tone and movement. This indicates that the action on the intestine is of the same nature as that of pilocarpine and muscarine. This action is in accordance with the repeated evacuation of the bowels seen in andromedotoxin poisoning. Repeated micturition also occurs, but I have not investigated the origin of this.

Uterus. Strips from sheep's uterus as obtained from the slaughter-house preserved in ice-cold Ringer were used in the same way as the intestine. A solution of 1:200,000 had no effect on the uterine contractions. Involuntary muscle supplied by the vagus appears to be affected more readily than other forms of unstriated muscle.

SECRECTIONS

Saliva. Salivation is observed in poisoning in sheep, in cats and dogs and to a lesser extent in rabbits and rats. Profuse salivation occurs in the first stages of poisoning, preceding the vomiting in time of appearance, and continues till the animal

becomes narcotised. But in experiments under anaesthesia salivation in any considerable degree was not observed, though stimulation of the chorda gave profuse secretion. It is therefore probably only reflex in origin and is only a preliminary to the vomiting.

The bronchial secretion as we have seen before, is also increased. It may be partly of the same nature as the salivation and possibly also in part at least due to stimulation of the vagus endings in the lungs.

Urine. The quantity of urine secreted did not seem to be influenced definitely in any one direction by sublethal doses administered to a rabbit day after day.

EXCRETION

De Zaayer and Plugge state that the poison is excreted in the urine. A rabbit, weight 1600 grams, was injected hypodermically on successive days with the doses increasing from 0.047 mgm. to 0.117 mgm., in all with 0.47 mgm. The collected urine was concentrated by evaporation in a vacuum, mixed with clean sawdust and extracted with chloroform in a Soxhlet apparatus; the residue was taken up in 1.35 cc. of water. 0.6 cc. of this injected into a frog of 17 grams, produced the symptoms of paralysis characteristic of the poison and killed the animal in between three and one half and nineteen hours. Taking this dose as equivalent to 3.0 mgm. per kilo (the fatal dose for frog) the total amount obtained from the urine is 0.144 mgm. out of 0.47 mgm. administered hypodermically. In other words, at least a third of the poison leaves the body in the urine in an uncombined state.

De Zaayer and Plugge mention references in the writing of Pliny (12), and Dioscorides to the effect that the poison of "Mad-honey" obtained from the flowers of rhododendron is excreted by the faeces at least in part, when it is administered per os. But I have been unable to detect the presence of andromedotoxin in the faeces of rabbits to which sublethal doses of poison were administered for several consecutive days either subcutaneously or orally.

CONCLUSIONS

1. Andromedotoxin, the active principle of the rhododendron, acts upon the terminations of the vagus which it first stimulates and then paralyzes; this explains some of its most prominent effects, which are:

a. An alternation of inhibition of respiration for short periods, and respiratory movements; slowing of the respiratory rhythm.

b. Dyspnoea of the asthmatic type due partly to stimulation of afferent fibres and partly to spasm of the bronchial muscle from stimulation of its motor nerve, the vagus.

c. Increase of bronchial secretion.

d. Slowing of the heart and fall of blood pressure followed by acceleration and rise of pressure.

e. Repeated evacuation of the bowels.

2. It paralyzes the motor nerve ends in striped muscle; while the paralysis is developing, the muscle and nerve are more easily fatigued, but regain their excitability after a period of rest. In a stronger concentration the poison also affects the muscle substance itself which thus loses its excitability permanently. The manifestation of this fatigue is seen best and earliest in the nerves and muscles which have to be constantly in action, viz., the phrenics and diaphragm.

3. Death from andromedotoxin occurs in two forms:

a. With very large doses it supervenes rapidly and is due to a direct action upon the heart, the ventricles being arrested in diastole or partial systole.

b. With smaller doses, it is due to failure of the respiration through a paralysis of the phrenics and often of the diaphragmatic muscle also.

4. There is a narcotic action upon the higher centers in the brain; the spinal cord is not affected.

5. It produces a condition of arrhythmia in the heart dependent upon a direct depressant action upon the conducting tissue between the auricle and ventricle leading to heart-block, or upon the excitability of the ventricle itself. The period required for diastolic relaxation of the ventricle is increased and the diastole is incomplete.

The perfused frog heart is arrested with the ventricle as well as the auricle in diastole; while in a frog injected with a fatal dose, the ventricle is arrested in total or partial systole with the auricles distended. The perfused mammalian heart is arrested in systole of the ventricles, but in death of an animal injected with the poison, the right side is distended and left empty or in partial systole.

6. The poison has a slight vaso-constrictor effect of peripheral origin upon the blood vessels.

7. Involuntary muscle which is not supplied by the vagus is not affected.

8. The seat of the emetic action of the poison has not been determined. The increased secretion of saliva is probably only the first stage of the emetic action and is not due to a specific effect upon the salivary glands or their secretory nerves.

9. At least a third of the poison injected hypodermically leaves the body unchanged in the urine.

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THE RELATION OF HISTAMINE TO INTESTINAL INTOXICATION

II. THE ABSORPTION OF HISTAMINE FROM THE INTESTINE

JONATHAN MEAKINS AND CHARLES ROBERT HARINGTON

Department of Therapeutics, University of Edinburgh

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In a previous paper (1) we have been able to demonstrate the presence of histamine in the contents of the human cecum and transverse colon in several cases. In some of these cases the histamine was still present after a period of weeks from the date of the operative relief of intestinal obstruction. The concentration of histamine observed was in all cases minute. These results would certainly indicate that histamine is normally present in the contents of the large intestine and that its presence there is not dependent upon the existence of an obstruction. If, therefore, histamine is to be regarded as an active agent in cases of intestinal intoxication, it is necessary to postulate either that the small quantity formed is quantitatively absorbed, or that, after absorption, the substance is not susceptible to oxidation in the body as are most compounds of a similar chemical nature.

In the attempt to obtain evidence upon this point we were led to undertake experiments on the absorption of histamine from different parts of the intestine.

Mellanby (2) in the course of numerous experiments upon the absorption of histamine from the intestine of cats found that the rate of disappearance of histamine from the intestine increases on passing down the small intestine towards the cecum. That the disappearance of histamine may be accounted for by absorption was indicated in certain experiments by a profound fall of blood pressure when this was recorded. He also states that he

found no evidence of disappearance or absorption of histamine from the cecum.

In the experiments about to be described we have confirmed Mellanby's results with regard to the relatively high rate of absorption of histamine from the ileum; but in addition we have obtained distinct evidence of its absorption from the cecum.

The experiments were carried out as follows: Cats were always used under paraldehyde anesthesia, a little ether being used in addition in the early or operative part of the experiment. Tracheotomy was first carried out; a cannula for the blood-pressure record was inserted in the carotid artery, and then the abdomen was opened and the part of the intestine to be experimented upon was isolated and tied off. A cannula was inserted into the loop of intestine for the injection of the solution of histamine; this eliminated the possibility of direct introduction of a trace of histamine into the circulation such as might occur during the piercing of the intestinal wall with the needle of the syringe. The cannula into the cecum was introduced through the ileocecal valve and the cecum ligated about it so as to prevent any chance of regurgitation into the ileum. In the case of female cats a tracing of the uterine contractions was taken in addition to tracings of respiration and blood pressure. In these experiments the animal was immersed bodily in a bath of saline at 36° to 37°C. and the movements of the uterus were recorded by means of the Cushny myocardiograph.

The histamine used was a solution of the acid phosphate in normal saline, containing 1 per cent of histamine. This solution was warmed to 37° before injection, the syringe and cannula being washed out, after the injection, with a few cubic centimeters of warm saline.

In table 1 will be found the results of the first series of experiments.

The following points call for special attention. In the first place the fall of blood pressure per minute is most rapid after an injection into the ileum (figs. 1, 2 and 3) slightly less so on introduction into the duodenum, and very slow after introduction of similar and larger doses into the stomach and cecum

TABLE I

REGION OF INTESTINE	NUMBER	SEX	PREGNANCY	WEIGHT grams	INITIAL DOSE mgm.	DOSE IN MILLIGRAMS WEIGHT IN GRAMS	ORIGINAL BLOOD PRESSURE mm. Hg	SHOCK FALL BLOOD PRESSURE	RECOVERY IN BLOOD PRESSURE	BLOOD PRESSURE AFTER 15 MINUTES	BLOOD PRESSURE FALL PER MINUTE	REMARKS
Stomach.....	I	M		1600	160	1:10	174	11	3	142	2.0	
	II	F.	+	2150	25	1:90	154	-	-	95	4.0	Uterine response
Duodenum...	III	F.	+	2800	50	1:56	142	-	-	92	3.3	
	IV	F.	+	2800	50	1:56	136	104	-	28	7.0	Uterine response and respiratory disturbance
Ileum.....	V	F.	-	2800	50	1:56	151	63	5	45	7.0	No uterine response. ? respiratory disturbance
	VI	F.	-	2300	50	1:46	156	24	-	86	5.0	? respiratory disturbance
	VII	F.	-	1800	40	1:45	133	57	12	72	4.0	
	VIII	F.	+	2900	100	1:29	157	-	-	106	3.5	Resistant cat
	IX	F.	+	2750	50	1:55	136	-	-	73	4.1	Uterine and ? respiratory response
	X	M.		2200	90	1:25	162	34	8	102	4.0	Respiratory disturbance
Cecum.....	XI	M.		1900	150	1:12.5	111	-	-	116	0.0	
	XII	F.	-	2300	50	1:46	184	-	-	108	1.0	
	XIII	M.		2400	50	1:48	136	-	-	130	0.4	
	XIV	M.		2500	200	1:12.5	125	28	19	120	0.33	
	XV	F.	-	2750	50	1:55	136	44	10	120	1.0	

(figs. 4 and 5). In the majority of cases the introduction of the histamine is followed, after a latent period of a few seconds by a sharp fall of blood pressure which is usually complete in fifteen to twenty-five seconds. This fall may be followed by a certain amount of recovery (figs. 2 and 5). As this delay or recovery passes off there then ensues a slow but gradual fall of blood pres-

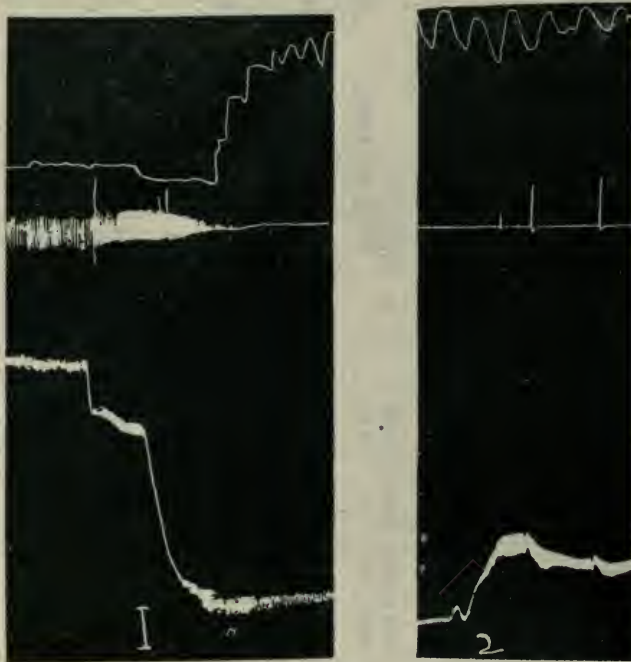


FIG. 1. Refers to experiment IV, table 1. Upper tracing = uterus; middle tracing = respiration; lower tracing = blood pressure. At 1, 50 mgm. histamine introduced into ileum. At 2, 10 mgm. histamine introduced into vein. Interval between 1 and 2 is fifteen minutes.

sure. These three phases correspond to those described by Dale and Laidlaw as the result of intravenous injections. The first two closely correspond in point of time, duration and degree to what these authors found except that they are slightly delayed. The third phase is much more gradual than when intravenous injections are made. In the case of the cecum the recovery may be almost complete (fig. 5) and the third phase

may not develop. In those experiments on the ileum where the sharp primary fall of blood pressure does not appear there is a steady decline of the blood pressure of practically the same order as that following the sudden primary fall (fig. 3). In the case of the cecum the steady fall of blood pressure is comparatively

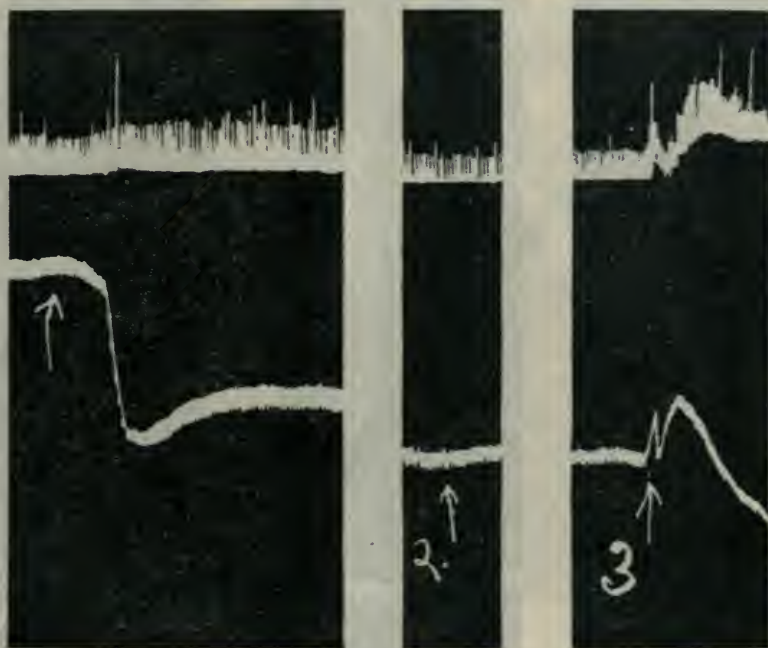


FIG. 2. Refers to experiment VII, table 1. Tracings of respiration (upper) and blood pressure. At 1, 40 mgm. histamine introduced into ileum. At 2, 10 mgm. histamine introduced into ileum. At 3, 10 mgm. histamine introduced into vein. Interval of fifteen minutes between 1 and 2; seven minutes between 2 and 3.

insignificant (fig. 4). It is probable that this sudden primary fall should be a constant feature of these experiments and that its absence in certain cases may be accounted for by the presence of masses of semi-digested food which delay the process of absorption. Among those cases where we washed out the gut before the injection we met with only one in which the sudden fall was absent.

It will be seen from these experiments on the ileum that results obtained under corresponding conditions are not constant. In fact they fall into three classes:

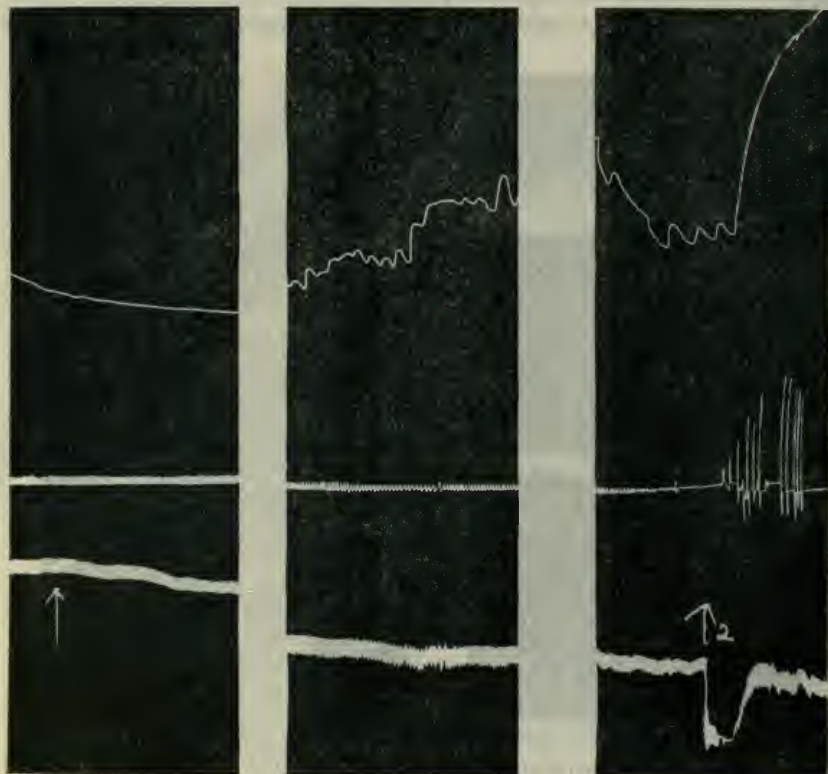


FIG. 3. Refers to experiment IX, table 1. Upper tracing = uterus; middle tracing = respiration; lower tracing = blood pressure. At 1, 50 mgm. histamine into ileum. At 2, 10 mgm. histamine into vein. Interval of twenty-seven minutes between 1 and 2.

1. Those exhibiting the sudden primary fall of blood pressure followed by a more or less pronounced recovery which merges into the gradual persistent blood-pressure decline (fig. 2). This type closely resembles the findings of Dale and Laidlaw (6) when they injected relatively large doses intravenously.

2. Those experiments showing the sudden primary fall with-

out rapid recovery (fig. 1). This type appears analogous to the effect found by Dale and Laidlaw (8) when relatively small doses were injected intravenously.

3. In contra-distinction to these authors' findings there was no evidence of eventual recovery but the blood pressure continued

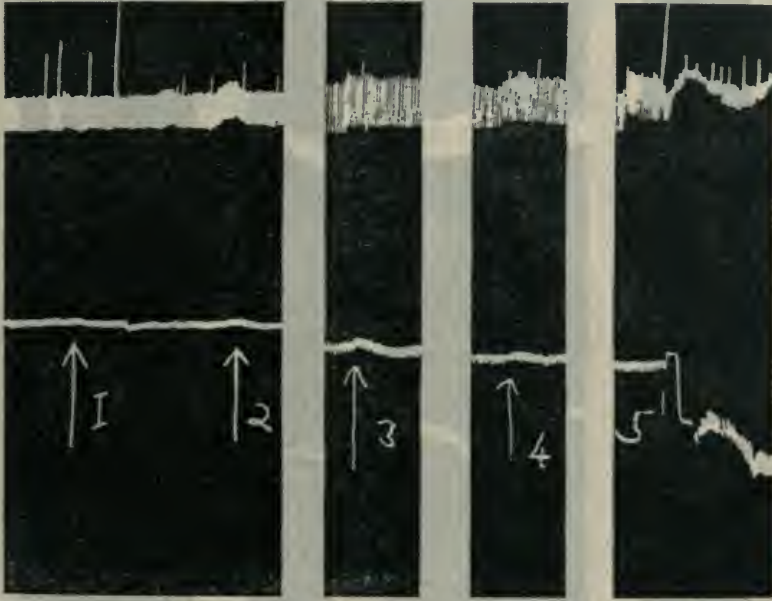


FIG. 4. Refers to experiment XIII, table 1. Upper tracing = respiration; lower tracing = blood pressure. At 1, 3 cc. saline into cecum. At 2, 50 mgm. histamine into cecum. At 3, 100 mgm. histamine into cecum. At 4, 100 mgm. histamine into cecum. At 5, 10 mgm. histamine into vein. Time intervals. twenty-five minutes between 2 and 3; twelve minutes between 3 and 4; eight minutes between 4 and 5.

slowly and steadily to fall. This suggests a close analogy to their findings when they introduced the histamine by a slow infusion. In such cases they obtained a rapid fall of blood pressure (without a temporary recovery) to be continued by a steady gradual fall.

The results obtained by us suggest strongly that the initial phenomena depend upon the amount of histamine suddenly

absorbed. This may apparently vary from a relatively large amount to a relatively insignificant dose but the gradual absorption brings about the persistent though slow fall of blood pressure. This would indicate that the rate of continued absorption was greater than that of destruction.

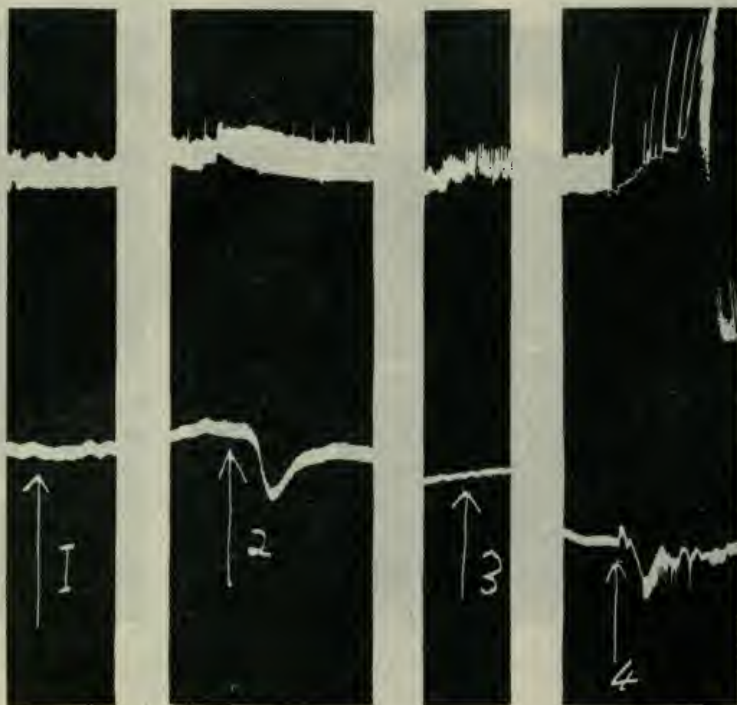


FIG. 5. Refers to experiment XIV, table 1. Tracings as in figure 4. At 1, 5 cc. saline into cecum. At 2, 200 mgm. histamine into cecum. At 3, 150 mgm. histamine into cecum. At 4, 10 mgm. histamine into vein. Time intervals: seven minutes between 1 and 2; twenty-three minutes between 2 and 3; fourteen minutes between 3 and 4.

It is also to be noted that, when the blood pressure is gradually falling, a further injection of histamine into the bowel does not reproduce the acute phenomena but leaves the steady continual fall of blood pressure unaffected (figs. 2, 4 and 5). An interesting point is the fact that intravenous injection of histamine subsequent to a shock produced by absorption from the gut,

instead of producing the usual fall of blood pressure, produces a considerable but transient rise of the latter (figs. 1 and 2). This result is analogous to those obtained by Dale and Richards (3) on adding histamine to the perfusion fluid of isolated organs in the absence of adrenalin or of red blood cells, and is probably due to the effect of the drag in constricting the arteries, unmasked by its effect as a capillary dilator.

It has been shown recently by Schenk (7) that the toxic effects of histamine are antagonised by simultaneous doses of adrenalin. The possibility here suggests itself that during slow absorption of histamine the store of adrenalin, rapidly mobilised to counteract the effect, becomes depleted, and hence at the time when we give the intravenous injection the conditions prevailing in the body are similar to those obtaining in Dale's experiments.

A number of cases of respiratory disturbances were observed during absorption (figs. 1 and 3). The earliest response was the development of the periodic type of breathing. If the respiratory effect became more pronounced it was manifested by a gradual diminution in the depth, with occasionally complete cessation of respiration. The uterus frequently showed an increase of tone particularly when pregnant (figs. 1 and 3).

Having thus shown that the absorption of histamine from any part of the intestine was a possibility we were next occupied with the problem of its subsequent fate after absorption, and more particularly with the question as to whether the liver played a part in its destruction.

In the cases of tyramine and indole-ethylamine, Ewins and Laidlaw (4) have shown that these substances, on perfusion through the surviving liver, are converted into the corresponding acetic acids, and that these acids are also found in the urine after oral administration of the respective amines. By analogy, therefore, one would expect histamine to be converted in the organism to glyoxaline acetic acid. Dale and Laidlaw (5) however state that in a few liver perfusion experiments they were unable to obtain any evidence of the destruction of histamine in this organ.

In the course of numerous liver perfusion experiments, an

account of which it is hoped to publish in a future communication, we have confirmed these results of Dale and Laidlaw. Like them, we found that the physiological activity of the perfusion fluid as measured by the amount of the latter required to produce a contraction of the isolated uterus of a guinea-pig, was somewhat increased after perfusion. We found however that the substance or substances which gave rise to this increased activity, could be destroyed by alkaline hydrolysis. After heating the perfusion fluid in the boiling water-bath for ten minutes with a concentration of 4 per cent of sodium hydroxide and subsequent cooling and neutralisation, the activity of the solution which had been perfused through the liver five times was found to be practically the same as that of the original fluid. It must however be borne in mind that although a positive result with a liver perfusion experiment may be taken as conclusive evidence of the possibility of such a change taking place in the animal organism, yet a negative result can not by any means be accepted as final. It is obvious that the conditions under which the liver is working during a perfusion experiment are excessively abnormal, however carefully the experiment may be carried out. Although, therefore, in view of the results of Ewins and Laidlaw with tyramine and indole-ethylamine, our experiments and those of Dale show that histamine is not readily oxidised in the liver, this is as far as we can go.

It occurred to us that a truer and more sensitive indication of the part, if any, played by the liver in the destruction of histamine, might be obtained by repeating our first absorption experiments with an Eck fistula in operation. By this means any histamine which might be absorbed would pass directly into the general circulation; the effect therefore should be far more prompt than normally if the liver be capable of destroying the amine. We therefore repeated a number of absorption experiments with an Eck fistula.

Our procedure for these experiments was as follows: The first part of the operation was carried out as described above for the simple absorption experiments. The inferior vena cava was clamped below the junction of the renal veins, ligatured at its

origin from the iliacs, and the intervening length stripped free and all small tributaries tied. The vessel was then cut above the ligature and washed out with citrated saline. As long a length as possible of the superior mesenteric vein was then stripped; if it were desired to divert the blood from the cecum or ileum the vessel was ligated immediately below its junction with the splenic vein. The superior mesenteric vein was then clamped as far down as possible, cut below the ligature and connected directly with the cut end of the inferior vena cava by means of the method described by Dale and Laidlaw (6). If the blood from the stomach and duodenum were desired to be diverted the superior mesenteric vein was ligated as low down as possible. It was clamped below its junction with the splenic vein. It was then cut immediately above the ligature and anastomosed with the inferior vena cava. The clamp was then removed and the portal vein ligated at its entrance into the liver. In this case therefore the blood flow through a short length of the superior mesenteric vein was reversed. The Eck fistula thus arranged functioned very satisfactorily in the case of the ileum and cecum but not so well in the case of the duodenum on account of the fall of blood pressure resulting from cutting off from the circulation such a large amount of the gut. This was not prevented by the primary removal of the latter.

The results of these experiments are set forth in table 2. It will be observed that the Eck fistula makes little or no difference in the case of the ileum and duodenum (figs. 6 and 7) but a conspicuous difference where the cecum is concerned (fig. 8). It will also be noticed that the rapid primary fall of blood pressure, following quickly upon the injection, is present in every case but one. The reason for the difference in the influence of the Eck fistula upon the toxic effects of absorption from the ileum and the cecum is not obvious. Mellanby, in the paper cited above, puts forward the suggestion that the absorption of histamine may take place largely by means of the lymphatic system. If this were the case our results would be explicable, owing to the fact that the ileum is much more liberally supplied with lymphatics than is the cecum, and therefore histamine from the former

TABLE 2

REGION OF INTESTINE	NUMBER	SEX	PREGNANCY	WEIGHT <i>grams</i>	INITIAL DOSE <i>mgm.</i>	DOSE IN MILLIGRAMS WEIGHT IN GRAMS	ORIGINAL BLOOD PRESSURE <i>mm. Hg</i>	SHOCK FALL BLOOD PRESSURE	RECOVERY IN BLOOD PRESSURE	BLOOD PRESSURE AFTER 15 MINUTES	BLOOD PRESSURE FALL PER MINUTE	REMARKS
Duodenum...	XVI	M.		3300	30	1:110	75	22	7	33 (10 min.)	4.2	Uterine response
	XVII	M.		2750	25	1:110	68	10	3	32	2.4	
Ileum.....	XVIII	M.		3450	100	1:34.5	138	46	4	68	5.0	Uterine response
	XIX	F.	+	2100	50	1:42	141	—	—	92	2.6	
Cecum.....	XX	F.	+	2400	100	1:24	154	68	—	30 (cat dying)	8.2	Respiratory arrest and uterine contractions
	XXI	F.	—	2500	100	1:25	145	69	34	105	2.6	
	XXII	F.	(re- cent)	2650	110	1:25	108	46	10	66	3.0	Respiratory and uterine response Vomiting and respiratory disturbance. Cat dies
	XXIII	M.		3000	110	1:28	102	50	22	48	3.5	
Ileum (small dose).....	XXIV	F.	+	2050	5	1:412	128	14	7	121	0.5	Uterine response

would not normally have to pass through the liver on its way into the general circulation. This would necessitate the assumption that histamine is absorbed from the cecum via the blood stream, and from the ileum via the lymphatic system. Thus the presence of an Eck fistula would greatly increase the amount of histamine absorbed directly into the general circulation from the cecum. Another point which might suggest the importance of

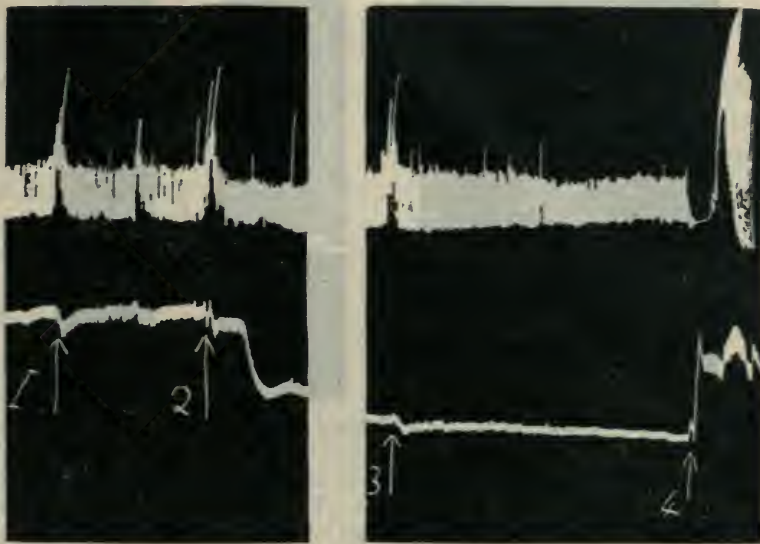


FIG. 6. Refers to experiment XVIII, table 2. Upper tracing = respiration; lower tracing = blood pressure. At 1, 5 cc. saline into ileum. At 2, 100 mgm. histamine into ileum. At 3, 50 mgm. histamine into ileum. At 4, 15 mgm. histamine into vein. Time intervals: ten minutes between 2 and 3; five minutes between 3 and 4.

lymphatic absorption is found in the observation made by Dale and Laidlaw (8) that there was an increased flow and concentration of lymph from the thoracic duct *after* the intravenous injection of histamine. It was so slight in amount that they accounted for it by the congestion and presumable rise of capillary pressure in the abdominal viscera. It seems to us however that this explanation cannot at present be accepted, since absorption by means of the lymphatics would be too slow to bring about the prompt fall of blood pressure which is observed in nearly

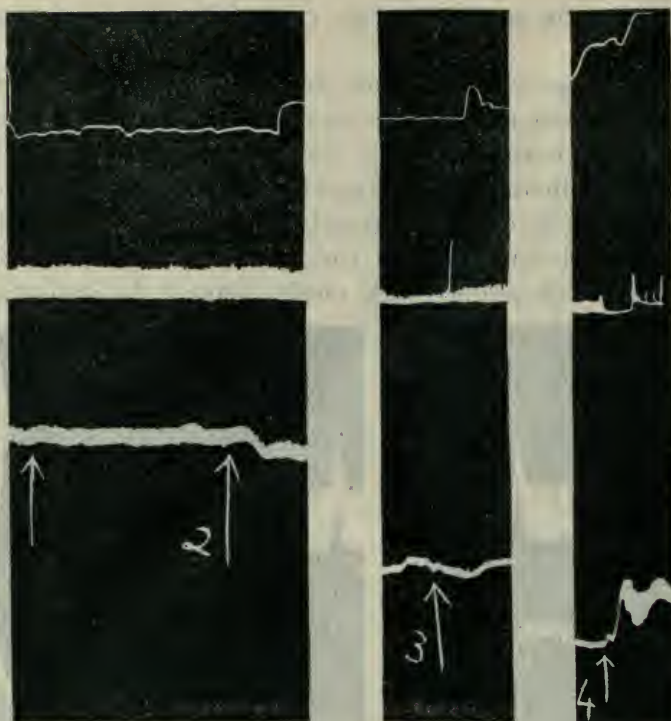


FIG. 7. Refers to experiment XIX, table 2. Upper tracing = uterus; middle tracing = respiration; lower tracing = blood pressure. At 1, 5 cc. saline into ileum. At 2, 50 mgm. histamine into ileum. At 3, 50 mgm. histamine into ileum. At 4, 10 mgm. histamine into vein. Time intervals: twenty-eight minutes between 2 and 3; twenty-eight minutes between 3 and 4.

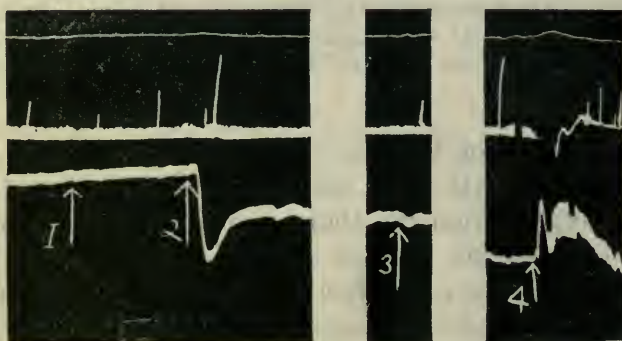


FIG. 8. Refers to experiment XXI, table 2. Tracings as in figure 8. At 1, 5 cc. saline into cecum. At 2, 100 mgm. histamine into cecum. At 3, 50 mgm. histamine into cecum. At 4, 10 mgm. histamine into vein. Time intervals: seven minutes between 2 and 3; thirty-two minutes between 3 and 4.

every experiment with the ileum. On the other hand the difference may be due simply to differences in the rate of absorption from the two regions of the intestine. We lack at present further definite evidence upon the point and are compelled to leave the question open.

In view of the striking difference in the results obtained in the cecum with and without an Eck fistula one is forced to think that the liver does play some part in the diminution of the toxic effects of absorption of histamine. This particular mode of expression is employed because it seems to us possible that the protective function of the liver may be multiple. Its power to effect chemical destruction of histamine is not as yet established, but in the "cushioning" effect of its mass of capillaries which prevents a sudden flood of the substance from reaching the general blood stream, a partial explanation of the phenomenon may be found.

In relation to the possibility of intoxication resulting from the presence of histamine in the intestine, there is a further point to be considered. It is conceivable that if the mucous membrane be damaged the rate of absorption of histamine may be affected. We therefore decided to try further absorption experiments after damage to the mucous membrane had been artificially induced. As a method of producing this damage we selected that of temporarily cutting off the arterial blood supply to the part under investigation, since we considered that this method was less likely to introduce disturbing factors than the use of some such substance as sodium fluoride for the purpose.

The results of our experiments by this method will be found in table 3.

The outstanding features are the very marked primary fall of blood pressure observed in every case but one, the lack of recovery after this fall and the fact that, after the primary fall, the further fall in blood pressure is extremely slight (figs. 9 to 12). This might indicate that under these conditions there was a preliminary absorption of a small amount of histamine followed by a condition in which absorption practically ceased. This would closely approach the result observed by Dale and Laidlaw

TABLE 3

REGION OF INTESTINE	NUMBER	SEX	PREGNANCY	WEIGHT	INITIAL DOSE	DOSE IN MILLIGRAMS	ORIGINAL BLOOD PRESSURE	SHOCK FALL BLOOD PRESSURE	RECOVERY IN BLOOD PRESSURE	BLOOD PRESSURE AFTER 15 MINUTES	BLOOD PRESSURE FALL PER MINUTE	REMARKS
(a) Cecum } (b) Ileum }	XXV	M.		3850	154 154	1:25 1:25	162 124	42 —	10 —	124 104 (10 min.)	2.3 2.0	Superior mesenteric artery clamped 5 minutes
(a) Cecum } (b) Ileum }	XXVI	M.		1700	68 68	1:25 1:25	140 118	23 43	13 —	118 64	1.5 3.6	Artery clamped for 11 min- utes
Ileum.....	XXVII	M.		3100	125	1:25	158	45	21	115	3.0	Artery off 15 minutes
Cecum.....	XXVIII	M.		1700	68	1:25	108	52	—	37	4.8	Artery off 15 minutes. Arti- ficial respiration
	XXIX	F.	—	1700	68	1:25	122	46	19	56	4.4	Artery off 15 minutes. Arti- ficial respiration
With Eck fistula												
Cecum.....	XXX	F.	—	2450	100	1:24.5	108	45	—	64	3.0	Artery off 20 minutes. No absorption of fluid
Ileum.....	XXXI	M.		3250	130	1:25	148	70	10	72	5.0	Artery off 15 minutes. No absorption. Dilution of 1 cc.



FIG. 9. Refers to experiment XXVII, table 3. (Artery clamped without Eck fistula.) Tracing of blood pressure. At 1, Clamp removed from artery. At 2, 125 mgm. histamine into ileum. At 3, 10 mgm. histamine into vein. Thirty minutes between 2 and 3.



FIG. 10. Refers to experiment XXXIX, table 3. (Conditions and tracing as in figure 10.) At 1, 68 mgm. histamine into cecum. At 2, 10 mgm. histamine into vein. Thirty minutes between 1 and 2.

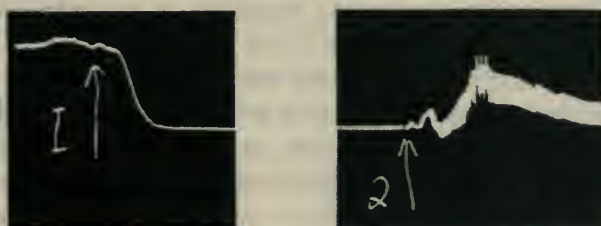


FIG. 11. Refers to experiment XXX, table 3. (Artery clamped and Eck fistula; tracing of blood pressure.) At 1, 100 mgm. histamine into cecum. At 2, 10 mgm. histamine into vein. Thirty minutes between 1 and 2.

after intravenous injection of a small dose of histamine. The Eck fistula, under these conditions, makes but little difference to the final result as measured by the rate of fall of blood pressure. There is, practically no absorption of fluid; in fact in one or two cases there seemed to be a dilution. (It must be remembered that the solution injected was distinctly hypertonic.)

The two cecum experiments which show an abnormal rate of fall of blood pressure are complicated by the fact that artificial respiration had to be maintained and hence are scarcely comparable with the other experiments.

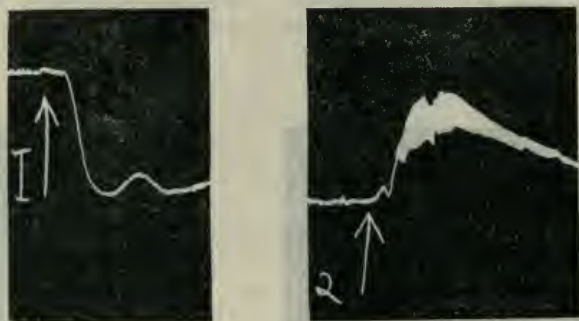


FIG. 12. Refers to experiment XXXI, table 3. (Conditions and tracings as in figure 11.) At 1, 130 mgm. histamine into ileum. At 2, 15 mgm. histamine into vein. Thirty minutes between 1 and 2.

DISCUSSION

The facts elicited by the experiments described above, together with the findings of histamine recorded in our previous paper, may help to throw some light upon the possibility of intestinal intoxication being caused by this substance.

Since we have been able to find no evidence of actual destruction of histamine in the liver, we are bound to assume that, if the substance is continually absorbed, it is likely to exert toxic effects upon the organism which would show themselves particularly in a lowering of the blood pressure. But, taking into account the very slow rate of absorption from the caecum and the minute concentrations in which we found it to be present, it is scarcely conceivable that any ill effects could be produced

by absorption from this part of the intestine. If, however, an appreciable concentration of histamine is formed for any reason in the ileum, we should expect, from the ease and rapidity of absorption from this region, to observe toxic effects. We might imagine such a condition of affairs arising for instance through incompetency of the ileo-cecal valve, which would permit of regurgitation of caecal contents into the ileum.

There seems to be no evidence that a damaged mucous membrane would facilitate a rapid absorption of histamine; in fact unless by some means a sudden flood of the substance were produced, we should expect the absorption to be slower than the normal. It is extremely unlikely, as Mellanby points out, that a high concentration of histamine should be produced in the bowel, owing to the intense peristalsis which it would evoke.

We consider therefore that the balance of the evidence so far obtained is against the view that histamine is an active agent in causing intestinal intoxication, except possibly in those cases where a definite structural deficiency, such as ileo-cecal incompetency, is involved.

As a result of these experiments it is clear that further work is necessary in at least two directions. In the first place the fate of histamine after absorption into the circulation must be determined. In the second the mechanical factors which may be involved in the absorption by the intestine must be more closely investigated. Experiments to determine both these points are at present being undertaken.

SUMMARY

1. The rate of absorption of histamine from the intestine, as measured by the rate of fall of blood pressure produced, is greatest from the ileum, somewhat less from the duodenum, and very much less, though still perfectly definite, from the caecum and stomach.

2. Absorption experiments with an Eck fistula in operation indicate that the liver exercises a protective function, probably more mechanical than chemical, against heavy doses of histamine.

3. With the mucous membrane damaged by cutting off the blood supply for five to fifteen minutes the fall of blood pressure would indicate that absorption takes place at first with a rush and then almost ceases.

We wish to express our indebtedness to Professor Cushny for the experimental facilities, and his criticism and advice.

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EVALUATION OF THE HORMONE OF THE INFUNDIBULUM OF THE PITUITARY GLAND IN TERMS OF HISTAMINE, WITH EXPERIMENTS ON THE ACTION OF REPEATED INJECTIONS OF THE HORMONE ON THE BLOOD PRESSURE

JOHN J. ABEL AND CHARLES A. ROUILLER

From the Pharmacological Laboratory of the Johns Hopkins University

It was stated in earlier papers (1, 2) that the addition of mercuric chloride or mercuric sulphate to a rather concentrated pituitary extract, as say a "20 per cent extract," precipitates the pressor and oxytocic hormone while depressor substances remain in solution. On decomposing the precipitate solutions were obtained which acted very powerfully on the guinea pig's uterus. The results indeed were so extraordinary that we preferred to withhold our tracings for the corroborations of later experiments. Circumstances beyond our control have prevented an earlier publication of our results.

In accordance with our directions raw material was very kindly gathered and prepared for us by Dr. Frederick Fenger of the Research Laboratory of Armour and Company and our thanks are due to both Dr. Fenger and Mr. F. M. Bell of this firm for the trouble they have taken and the care with which they have carried out our suggestions. The entire pituitary glands were placed in cold storage over night immediately after removal from the skull and on the following morning the posterior lobes were trimmed out from the congealed glands and ground up to a very fine paste. To 100 grams of this paste, 100 cc. of a 0.35 per cent solution of hydrochloric acid and 4 grams of powdered mercuric chloride were added and the whole mass was well shaken for a few minutes. A bottle of the proper capacity was then so filled with the mixture as to leave only a very small air space between the material and the tightly fitting cork.

The preparation was immediately sent to us by post and on its receipt in the laboratory was treated as follows: 10 grams of finely powdered mercuric chloride were added for each 100 grams of original posterior lobe paste. The cork was again replaced and the bottle was put in a shaking machine and gently shaken for two hours. It was then placed on ice over night. A heavy precipitate, which may be called the "proteid-mercuric precipitate," has now been deposited and this, as already stated, contains the pressor and oxytocic principles. This heavy precipitate, together with all of the insoluble portions of the posterior lobe, such as connective tissue and proteids, and also such other substances as are precipitable by saturation with mercuric chloride under the conditions above named, was collected at the pump and pressed to a very hard cake. The dry cake was then disintegrated by triturating it in a mortar with a saturated solution of mercuric chloride. The mass was again pressed to dryness and the dry cake was once more ground up in a mortar with a 1 per cent solution of mercuric chloride, pressed to dryness at the pump and set aside in a stoppered bottle. It may be remarked in passing that we have also tried to rub up the precipitate in a mortar with varying concentrations of alcohol. We finally desisted from this method of washing the cake as the alcohol seemed to carry away some of the pressor and oxytocic principles.

How this cake was further treated and its oxytocic power evaluated in terms of histamine phosphate is shown in the following description of one of our experiments (experiment III of the table).

The pressed cake from 100 grams of fresh glands weighed 48.8 grams. Of this cake, 20 grams, corresponding to 41.0 grams fresh glands, was ground up in distilled water, just neutralized to litmus with 2 per cent sodium hydroxide (the total volume of water and sodium hydroxide solution used was 100 cc.), treated with 2.5 grams powdered sodium chloride (the addition of salt was found to be necessary in order to produce coagulation of the mercuric sulphide precipitate), decomposed with hydrogen sulphide, filtered with suction, aerated to remove the excess of

hydrogen sulphide and treated with 2 per cent sodium hydroxide until the solution was still barely but distinctly acid to litmus (6 cc. of the alkali solution was required). The volume of the solution was now 98 cc. As the total volume of water and reagents used in working up the cake was 106 cc., 8 cc. of solution were lost in the various manipulations, the loss undoubtedly being due chiefly to the impossibility of squeezing the cake and precipitated mercuric sulphide (which was always more or less gluey in nature) perfectly dry after the decomposition with hydrogen sulphide. For purposes of calculation, it is assumed that each cubic centimeter of the solution is equivalent to $41.0/106 = 0.387$ gram fresh glands. Throughout all of the above operations the solutions were kept cooled with ice water.

One cubic centimeter of the solution thus obtained was diluted 10,000 times and 12 drops of the diluted solution were found to be equivalent in their action on the guinea pig's uterus, suspended in 40 cc. Locke's solution, to 10 drops of a 1:100,000 solution of histamine phosphate (fig. 1); 1 cc. of the original solution was therefore equivalent to 0.083 gram histamine phosphate and 1 gram of the fresh glands was equal in oxytocic power to $0.083/0.387 = 0.215$ gram histamine phosphate.

Ten cubic centimeters of the original solution, when evaporated in a platinum crucible and heated to constant weight at about 110° , left 0.3434 gram residue which was ashed with concentrated sulphuric acid, the ash thus obtained weighing 0.3742 gram. Assuming that the only inorganic matter in the original solution was sodium chloride, this 0.3742 gram ash (Na_2SO_4) would correspond to 0.3080 gram NaCl and hence the amount of organic matter in the 10 cc. of solution would be $0.3434 - 0.3080 = 0.0354$ gram or 0.00354 gram per cubic centimeter and therefore equivalent in oxytocic power to $0.083/0.00354 = 23.5$ times its weight of histamine phosphate.

A summary of our results is presented in the accompanying table.

Considering, first, the histamine phosphate equivalent of 1 gram of fresh glands, it will be noticed that different portions of the same lot did not give the same results. This is especially

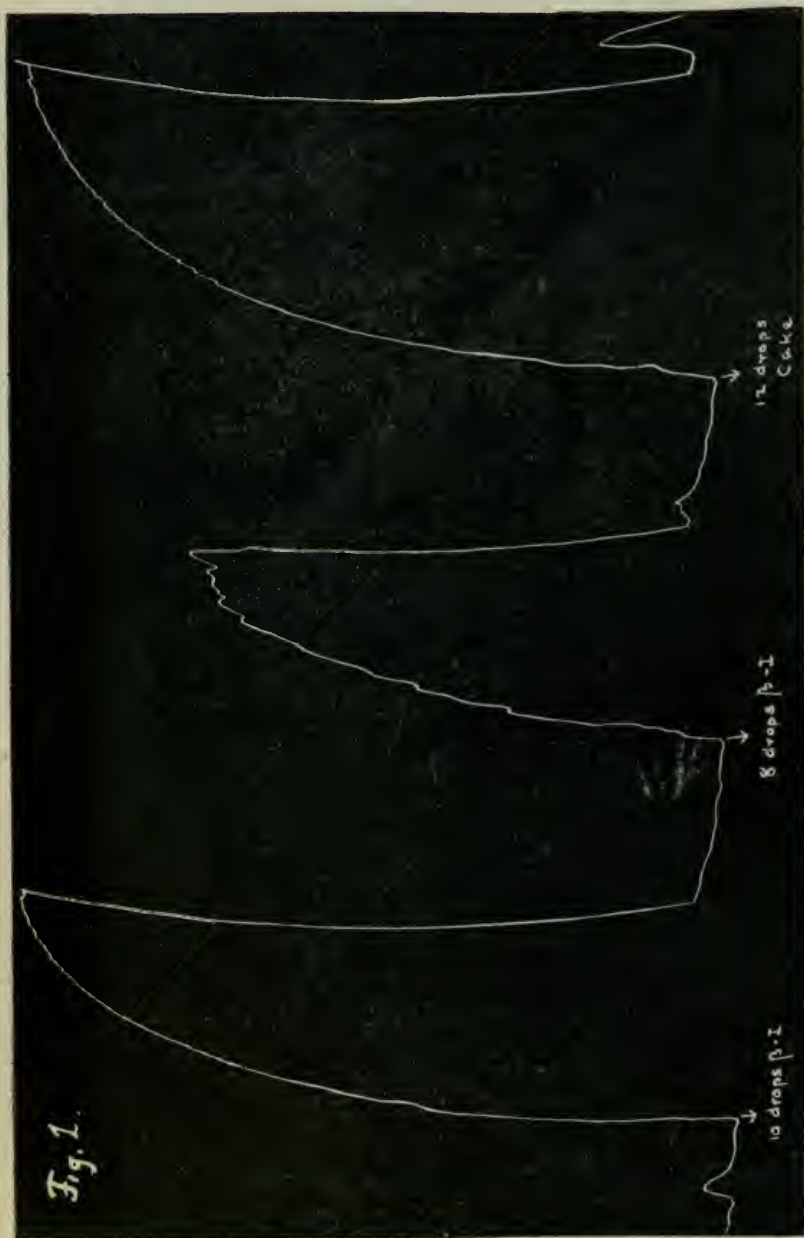


FIG. 1. COMPARATIVE ACTION ON THE GUINEA PIG'S UTERUS, SUSPENDED IN 40 CC. LOCKE SOLUTION, OF THE SOLUTION (0.000354 MGM. ORGANIC MATTER PER CC.) OBTAINED BY DECOMPOSING THE MERCURIC CHLORIDE CAKE (EXPERIMENT III) AND OF HISTAMINE PHOSPHATE (0.01 MGM. PER CC.)

Twelve drops of the solution of the decomposed cake is equivalent to 10 drops of the histamine solution; therefore the organic matter in the solution of decomposed cake is equivalent to $\frac{(0.01 \times 10)}{(0.000354 \times 12)} = 23.5$ times its weight of histamine phosphate.

TABLE I

	NUMBER OF EXPERIMENT*								
	I	II	III	IV	V	VI	VII	VIII	IX
Age of cake	2 days	14 days	6 days	46 days	2 days	22 days	7 months	3 days	8 days
Weight of cake used, grams	14	20	20	12.2	6.7	6.7	3.3	5.8	11.6
Equivalent in fresh glands, grams	27.5	39.2	41	25.5	10	10	5	10.5	21
Volume of solution, cc.	100	150	106	69	30	35	30	35	70
Fresh glands in 1 cc., gram	0.275	0.261	0.387	0.364	0.333	0.286	0.166	0.3	0.3
Organic matter in 1 cc., gram	†	0.00381	0.00354	†	0.00357	0.00423	†	†	0.00331
Histamine phosphate equivalent, grams, of									
1 cc. solution	0.05	0.08	0.083	0.03	0.075	0.05	0.0066	0.1000	0.1000
1 gram fresh glands	0.182	0.306	0.215	0.082	0.225	0.174	0.04	0.333	0.333
1 gram organic matter		21	23.5		21	12			30

*Experiments I and II were carried out with portions of a 51 gram cake obtained from 100 grams of fresh glands, III and IV with a 48.8 gram cake from 100 grams glands, V, VI and VII with a 73 gram cake from 110 grams glands and VIII and IX with a 63.7 gram cake from 115 grams. By "age" of the cake is meant the interval between the time the cake was first filtered off and pressed out and the day when the portion used for the experiment was decomposed.

† Organic matter not determined.

true of experiment III (0.215 gram) as compared with IV (0.082 gram) and of V (0.225 gram) compared with VII (0.040 gram). This may be due to the fact that the original cakes were not homogeneous, some portions containing relatively more of the inert, insoluble material of the gland and relatively less of the active principle precipitated by the mercuric chloride, but as the cakes had been thoroughly ground in a mortar with the solution or wash fluid before each of the three filtrations, it seems hardly likely that different portions of the cakes would differ greatly. A more probable cause of the observed differences in oxytocic power of different portions of the same cake is that, contrary to what we had hoped, the active principle is not stable over relatively long periods of time even when in the form of a (moist) mercury precipitate. Examination of the above table shows that, except in experiment II, the histamine equivalent per gram fresh gland decreased with the age of the cake. Experiment II is the only exception to this general observation but in this case the cake was only twelve days older than that used in I. This may be due to the 20-gram portion of the cake used for this experiment having been relatively richer in the active principle than the rest of the cake and thus really representing more than 39.2 grams of fresh glands, or to the uterine test being incorrect. That the last is not the case we are inclined to believe from the agreement in the histamine equivalent of the organic matter in this experiment with those found in III and V. Then, too, the active principle is so sensitive to the action of chemical reagents (acids, alkalies, etc.) and also, possibly, to atmospheric oxygen, that it is quite possible that it was injured to a greater or smaller degree in the preparation of the various solutions tested. [Since the above was written, we have decomposed five more portions of the cake used for experiments VIII and IX, with the following results:

Age of cake (days).....	21	28	32	36	39
Histamine phosphate equivalent of 1					
gram fresh infundibulum.....	0.25	0.10	0.055	0.133	0.083

These values, in connection with those for the 3- and 8-day old portions of the same cake, reported in the table, viz., 0.333 gram

histamine phosphate per gram of fresh posterior lobe, seem to leave no doubt that the hormone in the moist cake deteriorates very considerably on standing.] On the whole, we believe we are justified in saying that the organic matter in our solutions, though still contaminated with inert material, *was as powerful in its action on the guinea pig's uterus as at least twenty to thirty times its weight of histamine phosphate*, and that the hormone, when once isolated as a chemical individual, will prove to be, weight for weight, very many times more active than histamine.

DISTRIBUTION OF OXYTOMIC AND DEPRESSOR SUBSTANCES BETWEEN
THE PROTEID-MERCURIC CHLORIDE CAKE AND
THE FILTRATE FROM IT

Practically all of the oxytomic principle of the posterior lobe of the hypophysis is contained in the cake. The filtrate and wash liquids from the whole of the 51 gram cake used in experiments I and II amounted to about 200 cc. and were approximately equivalent in oxytomic power to a 1:10,000 solution of histamine phosphate, i.e., the whole filtrate from the 100 grams of fresh glands was equivalent to only 0.02 gram histamine phosphate, while the cake, from the results of experiments I and II, was equivalent to 18 to 30 grams. Similarly, the cake from 115 grams glands, used in experiments VIII and IX, was equivalent to 38 grams histamine phosphate and the filtrate to only 0.094 gram.

The work of Trendelenburg and Borgmann (3) tends to confirm the observation that our cake contains practically all of the oxytomic principle. They ground, separately, 12 ox posterior lobes with a little ignited sea sand, slowly adding 0.01 N hydrochloric acid until the mixture was 2 per cent with respect to the fresh glands, brought the mixtures to a boil, cooled and filtered them and tested the filtrates on the guinea pig's uterus. They found that the histamine hydrochloride equivalent per gram of fresh gland varied in the individual glands from 0.018 to 0.42 gram, the average for the 12 glands being 0.17 gram of the hydrochloride or 0.28 gram of the phosphate. The average obtained

by us for 149.2 grams glands in experiments I, II, III, V, VIII and IX is 0.26 gram of the phosphate per gram of fresh gland.

The filtrate from the cake contains all of the preformed depressor substance or substances which are normally present in extracts of the posterior lobe. The accompanying tracing (fig. 2) shows the effect of an intravenous injection of a small fraction of

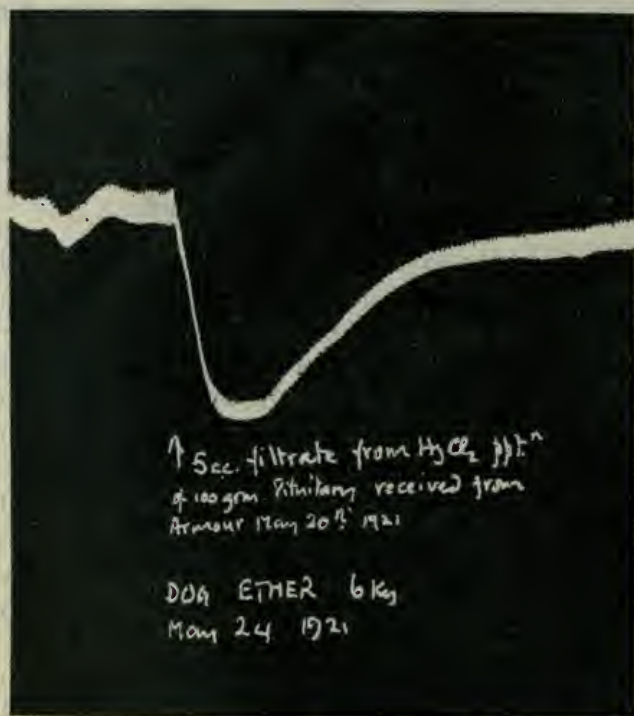


FIG. 2. EFFECT ON THE BLOOD PRESSURE OF A DOG OF AN INTRAVENOUS INJECTION OF A SMALL PORTION (ABOUT $\frac{1}{50}$) OF THE ENTIRE FILTRATE, AFTER REMOVAL OF THE MERCURY, FROM THE PROTEID-MERCURIC CHLORIDE CAKE FROM 100 GRAMS FRESH GLANDS

the filtrate from the proteid-mercuric chloride cake after removal of the mercury. Not only the *first* but all subsequent injections of the filtrate give this pronounced vaso-depressor action, whereas the first injection of the solution which is prepared from the cake itself *always* gives a clean-cut rise in the blood pressure, as was

abundantly shown also in earlier papers. *We have then a clean-cut separation of substances that act on the blood pressure. The cake yields the pressor principle while the filtrate contains only depressor substances.*

ACTION OF A PICRATE OF THE PRESSOR-OXYTOCIC PRINCIPLE ON
THE GUINEA PIG'S UTERUS

Further evidence for our belief that the pituitary hormone is many times more powerful than histamine is found in some experiments made by us in the fall of 1920. We may say at once that we have discarded the method used at that time in our attempt to isolate the active principle. We concluded that it involved such serious losses of the active principle as to be impracticable. The essential features, however, of the method are as follows: a 20 per cent extract in dilute acetic acid made from finely minced fresh posterior lobes was rapidly evaporated in flat bowls on the water bath under the electric fan. The residue was then taken up in a very little water so that a thickish mixture resulted. Into this mixture powdered anhydrous sodium carbonate was stirred, until it was certain that a large excess had been added. The mixture was then divided among a number of flat glass bowls, into each of which only a small quantity was ladled. The bowls were placed in vacuum desiccators charged with sulphuric acid, which were at once exhausted and twelve hours later the residues were found to be so thoroughly dry that they could be ground to a fine white powder. This powder was repeatedly extracted with dry chloroform for the removal of small quantities of histamine such as are always present in pituitary extracts that have been exposed to the action of weak acids and heat for a short time. After the removal of all traces of chloroform the dry powder was extracted with 95 per cent alcohol, gentle heat considerably below the boiling point of alcohol being occasionally applied to facilitate the extraction. The various alcohol extracts were combined and the alcohol driven off on the water bath at a low temperature under the electric fan. By this method one obtains a physiologically



FIG. 3. SHOWS THE POWERFUL ACTION OF THE PITUITARY PICRATE AS COMPARED WITH THE DIPICRATE OF HISTAMINE ON THE UTERUS OF THE VIRGIN GUINEA PIG

A half horn was suspended in 40 cc. of Tyrode's solution. At 1, a number of drops of histamine picrate were added so that the strength of the histamine picrate in the suspension chamber was as 1:100,000,000. At 2, the pituitary picrate was added in such amount that the Tyrode's solution contained the salt in the strength of 1:1,000,000,000. This strength of solution caused the uterus to pass into a condition of tetanic spasm of long duration.

highly active residue which is small in bulk as compared with the original material. An aqueous solution of this residue is markedly alkaline toward litmus paper as would be expected from the method employed. This material was dissolved in water and from the aqueous solution picrates and phosphates of high oxytocic power were prepared. Details need not here be given further than to say that fractional precipitation in various solvents as water and ethyl alcohol were employed in the separation of inactive picrates from the active ones. An active picrate was finally obtained which was not easily soluble in water and

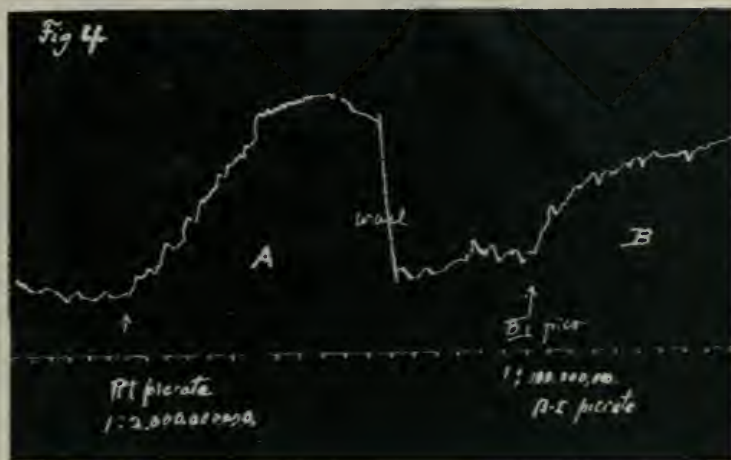


FIG. 4. ONE HALF HORN OF THE UTERUS OF THE VIRGIN GUINEA PIG IN TYRODE'S SOLUTION

At A, response to the pituitary picrate in the strength of 1:2,000,000,000. At B, response of the same uterus to the dipicrate of histamine in the strength of 1:100,000,000. It is seen that the response to the pituitary solution which is 20 times weaker than the histamine solution is decidedly more pronounced.

very little soluble in absolute alcohol. This picrate was turned into a phosphate which was extremely soluble in water, highly active for the guinea pig's uterus and exhibited marked pressor action on the blood pressure of dogs. We will present here only a few tracings (fig. 3 and 4) of the high activity of the picrate on the guinea pig's uterus as compared with the "dipicrate" of histamine. It will be seen that our picrate even in such high

dilution as 1,000,000,000 caused the uterus of the virgin guinea pig to pass into a condition of tetanic spasm of many hours' duration, as shown in A, figure 3. The action of this picrate in the very high dilution 1:2,000,000,000 is seen in A of figure 4, as compared with the decidedly weaker action of a histamine picrate in this concentration and shows that we had the good fortune to be using in our experiments an unusually reactive uterus. These experiments then show that it is possible to obtain a "salt" of the active principle, whose chemical purity is doubtful, which is at least twenty to thirty times (fig. 4) as active for the uterus of the guinea pig as is the corresponding salt of histamine.

ACTION OF REPEATED INTRAVENOUS INJECTIONS OF OUR SUBSTANCE ON THE BLOOD PRESSURE

Two years ago Abel and Nagayama stated that when the injections of their uninjured pressor salts were repeated too rapidly a slight fall of pressure occurred, or there might be only the slightest evidence of a rise of pressure. At the time they were inclined to believe that their observations proved that their pressor salts were still contaminated with the depressor substance of the gland. Later however they inclined "rather to the supposition that the uninjured pressor substance can *itself*, under the proper experimental conditions (previous injections, age of animal and stage of anesthesia), prepare the way for a decided fall of pressure in response to later injections." We have now found that a complete inversion in the blood pressure effect may be obtained on a second or third injection of the powerful pressor and oxytocic solution described above. It may be recalled that the organic matter of our solution is from 20 to 30 times as powerful a uterine stimulant as is histamine phosphate and that the intravenous injection of so small a quantity as 0.5 mgm. of this active organic matter into a dog will cause a pronounced and prolonged rise of blood pressure. The *first* injection of our solution *always* and quickly induces the sort of pressor action which is seen at 1 in figure 5. A second injection, when made after a certain interval, may cause only a small rise in the arterial pres-

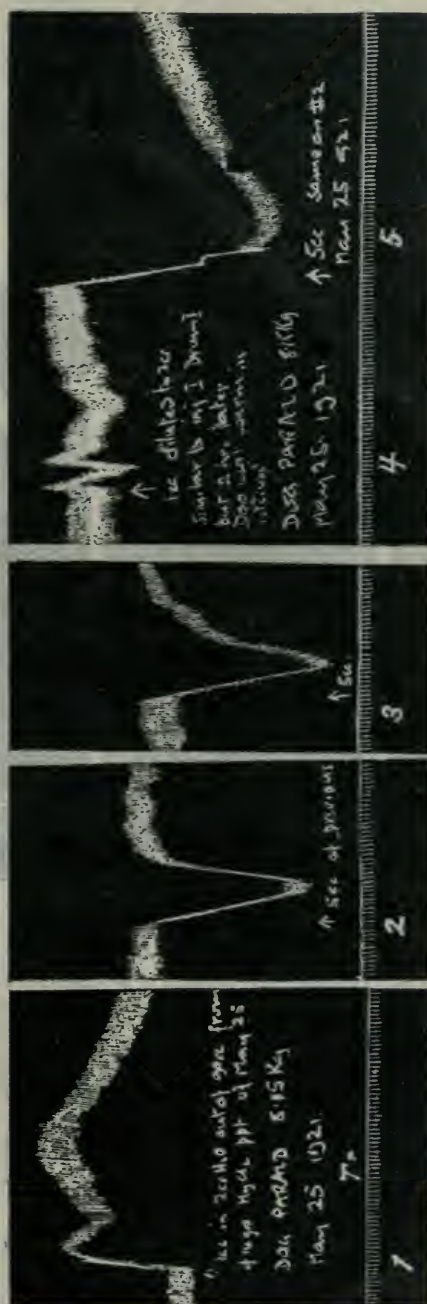


FIG. 5. EFFECT OF REPEATED INJECTIONS OF A SOLUTION OF THE PRESSOR-OXYTOCIC PRINCIPLE MADE FROM THE PROTEID-MERCURIC CHLORIDE-CAKE

Dog, weight 8.15 kgm. Anesthetic, paraldehyde. At 1, injected 1 cc. of extract containing approximately 3 mgm. of organic matter into the femoral vein. At 2, 6.6 minutes after injection 1, 5 cc. of the extract was injected. At 3, 4.6 minutes later again 5 cc. At 4, 2 hours later 1 cc. as at injection 1. At 5, 5 cc. was injected as at 4 and 2.

sure as seen at 2, figure 6, it may fail altogether to affect the pressure, or it may induce a *very pronounced fall in the arterial pressure*, as seen at 5, figure 5. In a word, a second or third injection may cause an actual inversion of the vaso-motor response. (See 2 and 3 and 5, figs. 5 and 3, fig. 6.)

This same inversion may be obtained with the salts of the pressor-oxytocic principle. This is shown in figure 7 which

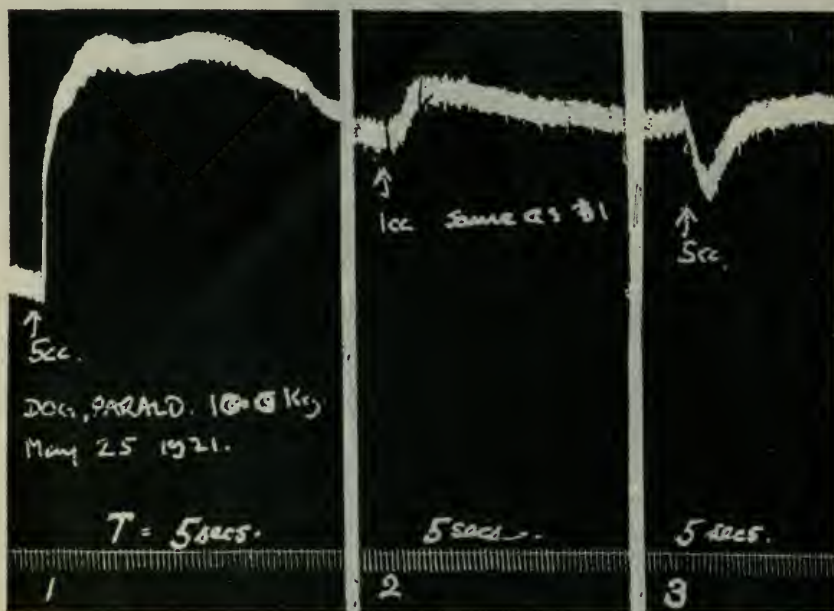


FIG. 6. SHOWS THE EFFECT OF REPEATED INJECTIONS OF THE SAME SOLUTION OF THE PRESSOR-OXYTIC PRINCIPLE USED IN THE INJECTIONS IN FIGURE 5

Dog, weight 10 kgm. Anesthetic, paraldehyde. At 1, injection into the femoral vein of 5 cc. of the solution. At 2, twenty minutes later, while the pressure was still very high, 1 cc. of the same solution was injected. At 3, 4.5 minutes after the injection at 2, 5 cc. of the solution was again injected. In this particular animal the inversion effect of the later injection at 3 is much less marked than in figure 5.

illustrates the effect of a second and a third injection of a pressor phosphate whose oxytocic value was five times that of histamine acid phosphate. This particular tracing is here given to show that this inversion of the vaso-motor effect in response to a second injection can hardly be due to inclusion of a separate

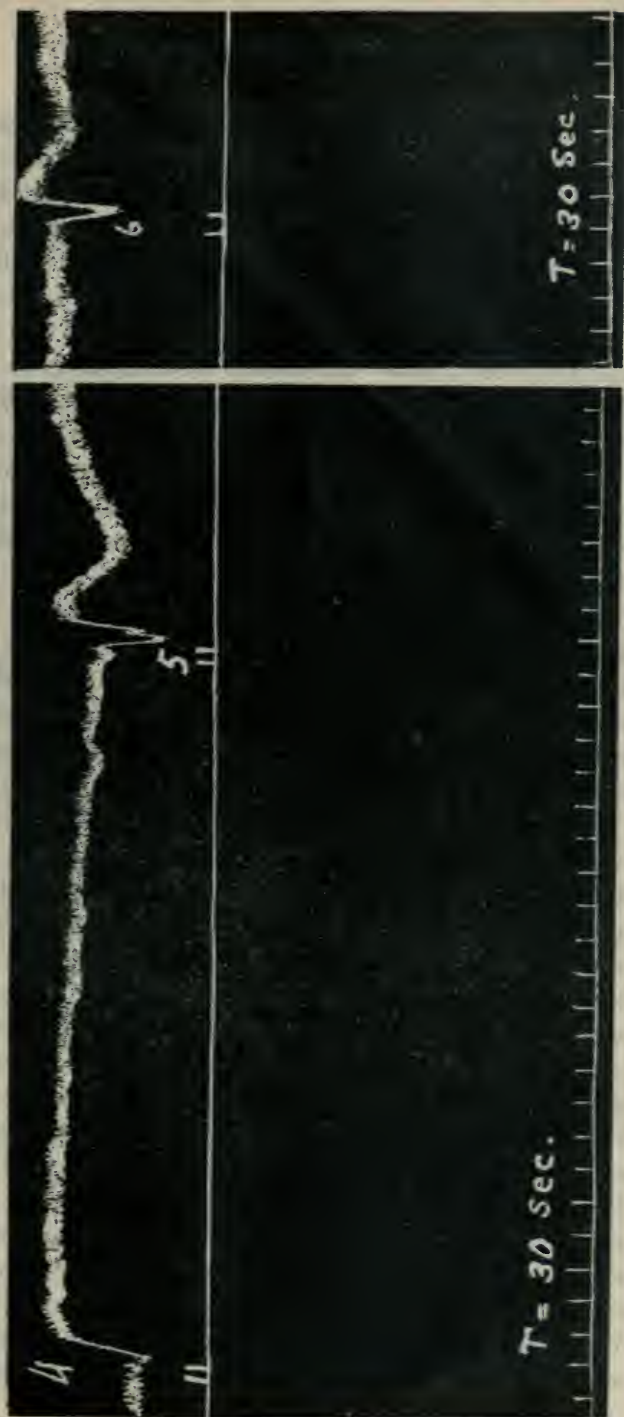


FIG. 7. SHOWS EFFECT OF FIRST, SECOND AND THIRD INJECTIONS OF A PRESSOR PHOSPHATE

Dog; fox terrier; weight 6.4 kgm; age about ten months; anesthetic, ether. At 4, injection into femoral vein of 1 cc. which equals approximately 0.5 mgm. pressor phosphate. At 5, injection of 4 cc. of same solution which equals approximately 2 mgm. of the phosphate. At 6, injection of same dose as at 5. The interval between 5 and 6 was sixteen minutes; that between 4 and 5 was 10 minutes.

depressor substance with a pressor substance. It is extremely unlikely that two principles, one a pressor and the other a depressor substance, should be present in both the original solution and the salts prepared from it, in such a nicely balanced ratio, as always to give only a *rise* of the arterial pressure on the first injection and a *fall* of pressure, or practically no effect at all, on a later injection. Everything in our experience points to the conclusion that the pressor substance in the infundibulum is the oxytocic principle, in other words that the two properties belong to one and the same substance. Howell (4) was the first to observe in 1889 that repeated injections of the ordinary unpurified extracts of the infundibulum may be less marked or fail altogether in raising the arterial pressure, the result depending, as he stated, upon the interval of time between injections and upon the strength of the dose used, but no one, to our knowledge, has hitherto obtained the results here described, more especially the inversion effect, with nearly pure solutions, or, more accurately speaking, with solutions of an extremely high oxytocic value and quite free from all traces of the preformed depressor substances always present in the ordinary infundibular extracts.

DISCUSSION

We have shown, by repeated experiments in this laboratory during the past two years, that it is possible to prepare, by a very simple method, an aqueous solution of the pressor and oxytocic principle of the pituitary gland, the organic matter of which is *actually equal in oxytocic activity to from 21 to 30 times its weight of the acid phosphate of histamine, or 12 to 18 times its weight of histamine dihydrochloride*. As the active principle in our solutions is probably present as a hydrochloride, the comparison with the hydrochloride of histamine is perhaps fairer than that with the phosphate.

Chemical manipulation of these highly active solutions with the aim of isolating the active principle in pure form meets with almost insuperable difficulties. These difficulties do not lie in the removal of the inert material but in keeping the active principle from being injured during the process. For example we

subjected our powerfully oxytocic solution (containing only about 3 mgms. organic matter per cubic centimeter) to a precipitation with mercuric acetate 3 times in succession. The mercury was removed from the precipitate with hydrogen sulphide in the usual way. Alcohol was employed to extract the active principle after concentrating the mercury-free solutions. Our final product, in the form of a phosphate, although apparently free from inert material, was now entirely devoid of a pressor action and had, in fact, been converted into a blood-pressure lowering agent and its oxytocic value had been reduced from 23 times to 1 time that of histamine phosphate. An intravenous injection of 10 mgm. of this blood-pressure lowering phosphate into a decerebrate dog weighing 10 kgm. also gave a very good bronchoconstriction.

Our attention now centers on this problem of isolating the pressor-oxytocic substance without loss of physiological activity or alteration in its properties. We have had in our hands at various times in the past two years pressor "salts" of the active principle which varied in oxytocic activity all the way from $\frac{1}{15}$ to 20 times that of a corresponding salt of histamine (figs. 3 and 4). They all gave the Pauly and biuret reactions, yet none of these salts could be called a pure chemical individual. In this connection we feel that it may justly be demanded of any one claiming to have isolated "pure crystalline" principles from the pituitary that he at least evaluate their oxytocic power in terms of histamine or some other unvarying standard. We confidently believe that the entirely uninjured principle will be found to be at least forty to fifty times more powerful than histamine in its action on the uterus of the virgin guinea pig. Quite aside from the results which future researches may establish, the quantitative measurements which we ourselves have given in the present paper prove quite conclusively that the posterior lobe of the pituitary gland contains what is by far the most powerful oxytocic principle now known, to mention only the most striking among the properties of this remarkable substance.

We have lately succeeded in reprecipitating the hormone with a mercurial salt apparently without loss of its physiological

properties and with a decided diminution of inert contaminating substances. A second precipitation yielded a solution, the *dry* matter (organic and inorganic) in which was at least *fifty-five* times as active for the guinea pig's uterus as an equal weight of histamine phosphate. Taking 0.4 gram as the average weight of the posterior lobe of the hypophysis of the ox (Hous-saye) and 0.28 gram histamine phosphate as the oxytocic equivalent of 1 gram of the fresh lobe (Trendelenburg and Borgmann), and assuming, in agreement with our finding above, that the hormone is equivalent to at least 55 times its weight of histamine phosphate, then the posterior lobe would contain not more than 2 mgm. of the hormone. As we proceed with the purification and isolation of the hormone, an objective which now seems attainable, we hope to determine the hormone content of the gland with greater accuracy.

The experiments which we have made during the past two years lead us to hold to the correctness of the conclusion stated two years ago by Abel and Nagayama, that there is but one specific hormone in the infundibulum and this substance has both vaso-motor and uterus-stimulating properties. Guggenheim (6) maintained in 1914 that the behavior of active pituitary solutions to alkalis is alone sufficient to prove that both physiological actions are properties of one and the same substance.

It may also be stated that in our opinion this substance also acts powerfully on the kidneys. We have during the past two years on a number of occasions injected intravenously small quantities of our highly active pressor salts into rabbits whose kidneys were actively secreting at the time, with the result that the urinary flow was either promptly diminished or entirely abolished. The uninjured pressor and oxytocic principle in small doses has no action on the bronchi (5). After very large doses there is only a slight indication of a broncho-constrictor effect, and, as we have seen again in recent experiments, this may be followed by an unmistakable broncho-dilator action. Our work on the broncho-motor action of the principle is, however, far from being complete. Here also, as in the case of the vaso-motor action, the effect of repeated injections of varying

doses must be studied. The broncho-constrictor action of the "inverted" hormone however is quite unmistakable. We are continuing our researches on the chemical nature of this active principle of the infundibulum whose great potency as an oxytocic agent in particular has been demonstrated in this paper.

We desire to express our great indebtedness to Dr. E. M. K. Geiling and Mr. V. Vermooten for much kind assistance in making numerous uterine and blood-pressure tests, the results of only a few of which could here be published.

SUMMARY

1. A method has been described which yields a preparation of the pressor-oxytocic principle of the infundibulum, which is actually equal in oxytocic activity to from 20 to 30 times its weight of the acid phosphate of histamine or from 12 to 18 times its weight of histamine dihydrochloride. It is estimated that when the active principle is freed entirely from the accompanying inert material it will be found to be weight for weight 40 to 50 times more powerful in its action on the guinea pig's uterus than histamine. This estimate is made on the assumption that the isolation of this unstable hormone as a chemical individual can be effected with the retention of its peculiar powers as manifested in the preparations described in this paper. In agreement with our findings as given above it appears that the hypophysis of the ox, the posterior lobe of which weighs on the average 0.4 gram, does not contain more than two milligrams of the oxytocic principle.

2. The powerful solution with its extremely low content in organic matter, which is obtainable by our method, exhibits *all* of the really characteristic physiological activities of ordinary saline extracts of the infundibulum. A first intravenous injection is always followed by a pure pressor vaso-motor response; a later injection by a pronounced depressor vaso-motor response, although the response to the later injection may be very slight. The actively secreting kidney of the rabbit responds to an injection by a diminished secretion or by an entire inhibition of the urinary flow.

3. The results obtained with our relatively pure solutions and with the salts obtained from them lead us to believe that the vaso-motor, oxytocic and renal action of our preparations are only the expression of the manifold physiological properties of one and the same hormone. In addition to this principle the infundibulum also contains depressor substances which have been described in previous papers. The methods described in this paper effect a clean separation of the pressor-oxytocic substance from these depressor substances.

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QUANTITATIVE STUDIES IN CHEMOTHERAPY. VI
RATE OF EXCRETION OF ARSENICALS, A FACTOR
GOVERNING TOXICITY AND PARASITICIDAL
ACTION

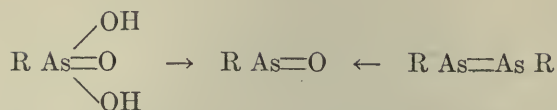
CARL VOEGTLIN AND J. W. THOMPSON

*Division of Pharmacology, Hygienic Laboratory, United States Public Health
Service*

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The rate of excretion of arsenic administered to animals and man in the form of inorganic or organic arsenic has received considerable attention, particularly since the introduction into therapeutics of arsphenamine and its derivatives. It is a surprising fact, however, to find that no attempt has been made heretofore at a correlation between the rate of excretion on the one hand and the toxicity and parasiticide action on the other. Yet this relation is, as will be shown, of the greatest interest from a theoretical as well as a practical aspect. In planning this work we were guided by observations described in previous papers of this series. For instance, due consideration was given to the fate of the various forms of arsenic in the animal body. Thus it was shown by Voegtlin and H. W. Smith (1920) that from a biological point of view the arsenicals fall roughly into three groups, viz.: (1) Arsenicals containing the arsenic in the pentavalent form, (2) Arsenicals of the trivalent "arsenoxide" type; and (3) Arsenicals of the arsenobenzene type.

Differences in the toxicity and parasiticide action of these types have been explained on the basis that they must be changed within the body into one type, namely, the trivalent oxides before they are capable of exerting their toxic action upon the cells of the host and on the parasites. This is illustrated by the following formulae:



in which R represents either a hydroxyl group or an organic radical. Representatives of these three types of arsenicals were therefore selected in sufficient numbers to justify general conclusions. The data relating to the toxicity and trypanocidal action were taken from papers by Voegtlin and H. W. Smith (1920, 1921). This made it necessary to use for the study of the excretion albino rats, as the toxicity and trypanocidal action had been determined in this species.

EXPERIMENTAL

As no reliable methods exist for the quantitative estimation in the excreta of the various metabolism products of organic arsenicals, it was necessary to confine the work to the estimation of the total arsenic, a procedure which, although it yields not a complete picture of the metabolism of arsenicals, does furnish information which, for reasons to be discussed later, is of great importance.

Even the estimation of the total arsenic requires a very accurate and sensitive method and taxes the carefulness of the experimenter to the utmost, as work with animals of the size of rats obviously does not permit the collection of large specimens.

The procedure was as follows: Healthy non-pregnant albino rats weighing approximately 120 grams were injected intravenously between 9 and 10 a.m. with the arsenical to be studied. The drug was used uniformly as an aqueous solution of the sodium salt. The dose of arsenic given was 3 cc. of a 1:100 arsenic equivalent solution per kilo body weight in every case. This arbitrary dose was chosen as being the average minimum effective dose of arsphenamine, the most important drug of this series. The data to be presented therefore relate to the relative rate of excretion of the amount of arsenic injected in different combination.

Immediately after the injection of the drug the animals were put into specially constructed small metabolism cages, provided with a glass funnel for the separate quantitative collection of urine and feces. Six hours after the injections the urine secreted in this period was collected by gentle pressure of the bladder region the urine thus obtained being added to the sample found in the collecting bottle and the washings from the cage and funnel. At the expiration of another eighteen hours another sample of urine was obtained in the same manner. The two samples together therefore represent the twenty-four-hour urinary excretion. The animals were then chloroformed, and the intestinal contents were added to the feces found in the cages at the end of the twenty-four-hour period. This material, consisting of intestinal contents and feces, will be referred to as feces.

Not less than five rats were injected with each drug, as we expected to find a considerable variation in the rate of excretion of the arsenic in different individuals and as it was essential to obtain reliable averages for comparison with the toxicity and trypanocidal action.

For the determination of the arsenic the samples of urine and feces were ashed according to the method of Gautier and Clausmann (1917) in which the material is first charred in a hot air oven, then carefully powdered and mixed with 2 or 3 per cent of its dry weight of potassium carbonate. This mixture was then transferred to an electric furnace, heated to such a temperature that the inside bottom of the furnace was a dull cherry red (250° to 300° C.). Under these conditions there was no loss of arsenic due to volatilization, and in one to four hours there was left a white or grayish white ash. This ash was thoroughly pulverized, then extracted repeatedly with hydrochloric acid (1:3), and finally sufficient hydrochloric acid (1:3) was added to make up to a volume of 50 to 100 cc., according to the probable arsenic content of the sample. Of this solution aliquot portions were taken for the Gutzeit test, which had been shown by Sanger and Black (1907), R. C. Smith (1912), Vinograd (1914), Collins (1918) and by us to yield reliable figures.

The apparatus used consisted of wide-mouthed generating bottles of 100 cc. capacity, each generator being connected to two upright tubes 7.5 cm. long and 1 cm. wide, and connected with each other by a rubber stopper. Both tubes were loosely packed with absorbent cotton moistened with a 5 per cent solution of lead acetate. Connected by a rubber stopper with the second upright tube, was a tube 12 cm. in length and 3 mm. internal diameter, containing the mercuric bromide test strip. These test strips were prepared from heavy, close-textured drafting paper, cut into strips 2.5 mm. by 12 cm. The strips were soaked for one hour in a 5 per cent alcoholic solution of mercuric bromide. On removal of excess solution the strips were separated and dried on a series of glass rods.

After transferring aliquot portions of the extract of the ashed sample to the generator, the latter was then three-fourths filled with hydrochloric acid (1:3), 10 to 15 grams arsenic free zinc were added and the apparatus connected immediately with the upright tubes. The generators were allowed to run until the hydrogen evolution had almost ceased, usually two hours. The test strips were then compared with standard strips. These standard strips were prepared by dissolving 4.948 grams freshly sublimed arsenious oxide of known arsenic content in sufficient sodium hydrate, then rendering slightly acid with sulfuric acid, and diluting to 1 liter. Of this tenth normal solution, 1 cc. was diluted to 1 liter, forming a stock solution containing 0.000005 gram arsenic per cubic centimeter. Amounts of this final dilution were taken corresponding to the following quantities of arsenic: 1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5 micromilligrams and treated in the generator as previously described.

All reagents and water used were arsenic free and arsenic contamination of the specimen was carefully avoided.

For reasons to be discussed later arsenic determinations were also made of the blood serum at various periods following the intravenous injection of the arsenicals. The blood was obtained by decapitation of the rats and collection of the blood in small, dry flasks provided with a few glass beads. The defibrinated

blood was immediately centrifuged at high speed and the serum separated from the corpuscles. Measured volumes of serum were ashed and treated in the above described manner for the estimation of total arsenic.

In control experiments with healthy rats of the same weight but which had not received any arsenic, it was shown that the normal physiological arsenic of the feces and urine was such an infinitely small amount that it could not possibly affect the results. In order to check the method still further it was shown that the arsenic recovered by ashing the whole rat immediately after the injection of the drug was identical with the arsenic injected.

The results of the analyses and their bearing on the toxicity and trypanocidal action are illustrated by the tables and charts. The minimum effective dose (M. E. D.) and minimum lethal dose (M. L. D.) is expressed in the tables as the number of cubic centimeters of a 1:100 arsenic equivalent solution per kilo body weight. This otherwise expressed means a 1:100 molecular solution of the pentavalent arsenicals and the drugs belonging to the trivalent arsenoxide type, and corresponds to a 1:200 molecular solution in the case of the arsenobenzene compounds. The charts were prepared by taking the toxicity of methylarsenious oxide and the effectiveness (trypanocidal action) of m-amino-p-hydroxyphenylarsenious oxide as 100 per cent respectively in relation to the toxicity and effectiveness of the other compounds. In other words the most toxic and most trypanocidal drug was taken as a standard for comparison. The blocks representing the excretion are the actual percentages of the injected arsenic.

DISCUSSION

The first point of interest brought out by this work is the fact that the rate of excretion of arsenic in different individuals injected with the same amount of a given drug under exactly identical conditions shows considerable variations which are beyond the errors attributable to the technic and the analytical method used (see table 1). It is significant that similar varia-

tions exist in the toxicity and parasiticial action in different individual animals of the same species. We believe that the variation in the rate of excretion in different individuals is one of the important factors which regulates the toxicity and parasiticial action. An individual which retains the arsenic longer than another one will obviously be exposed for a longer time to the action of the drug or its toxic metabolism product. This explains

TABLE 1

Variation of arsenic excretion (in per cent of injected arsenic) in different individuals

SODIUM SALT OF	URINE		FECES	TOTAL EXCRETION
	6 hour period	18 hour period	24 hour period	24 hour period
$R \cdot As = O$				
Arsenoxide.....	1.79- 6.67	5.56-12.55	4.51-10.30	17.09- 18.70
Methyl arsenious oxide..	3.65- 5.00	3.90- 8.19	1.15- 3.33	9.10- 13.88
Arsenious oxide.....	11.04-14.53	0.32- 1.33	3.47-13.16	18.55- 25.61
$R \cdot As = As \cdot R$				
Silver arsphenamine....	14.82-18.52	4.33- 7.01	13.58-17.68	35.73- 39.91
Arsphenamine.....	5.10- 7.81	4.31- 6.56	75.92-89.41	86.22- 98.82
Neoarsphenamine.....	26.00-40.47	16.67-44.67	26.09-49.00	80.83- 93.53
$ \begin{array}{c} \text{OH} \\ \diagup \\ R \cdot As = O \\ \diagdown \\ \text{OH} \end{array} $				
Arsenate.....	37.96-57.29	2.81- 9.23	1.08- 3.40	44.17- 63.23
Oxy-amino.....	72.96-87.50	4.03-17.16	4.03-12.09	57.45- 95.84
Arsacetin.....	82.04-91.11	3.10- 6.97	2.46- 7.74	92.88- 98.08
Atoxyl.....	81.27-88.32	4.89-12.51	4.18- 9.75	95.97- 99.33
Phenylglycin.....	66.00-89.90	7.32-31.60	2.60- 3.66	91.00-100.00

the necessity of using at least five rats in the official method of testing the toxicity of commercial arsphenamine and allied arsenicals. There is no reason to doubt that similar differences in rate of excretion, toxicity and therapeutic action exist in patients treated with arsenicals. In fact a generally accepted rule cautions against the use of arsenicals in cases with impaired kidney function. The data here reported seem to indicate that variations in toxicity and therapeutic value probably occur even in cases with *normal* renal function, and may

account for some of the toxic reactions sometimes observed in patients exhibiting no manifestations of renal diseases. Of further interest is also the fact that the variations in the rate of excretion of the arsenic of neoarsphenamine are far greater than those met with in the case of arsphenamine.

The influence of raising the dose of injected drug on the percentage excretion was studied only with p-amino-phenylarsonic acid. Here it was found that the rate of excretion was the same when the dose was increased ten times (30 cc. of a 1:100 arsenic equivalent solution per kilo body weight).

The comparison of the rate of excretion of the arsenic of the drugs belonging to the three biological groups (trivalent arsenoxides, arsenobenzene derivatives and pentavalent arsenicals) reveals the important fact that the trivalent arsenoxides show the slowest rate of excretion (see table 2 and Charts 3 and 4). They also are the drugs most toxic for the tissues of the host (organotropic) and the parasites (parasitotropic). The arsenobenzene derivatives show a much greater rate of excretion and the pentavalent arsenicals as a general rule with exception of arsenic acid, are excreted most rapidly. The relation between rate of excretion, toxicity and parasitical action (effectiveness) of the various drugs is brought out very clearly in table 2 and charts 1, 2, 3, and 4. In charts 3 and 4 the total twenty-four-hour excretion is compared with the toxicity and the relative trypanocidal action (effectiveness). It should be emphasized that these two charts do not give the truest picture of this relationship for the reason that the variation of the rate of excretion of the different compounds is actually more marked than would appear from an examination of these charts. This is due to the fact that most of the arsenic of the pentavalent organic arsenicals is exceedingly rapidly excreted, as shown by charts 1 and 2. For instance the percentage of injected arsenic appearing in the urine within the first six hours is as follows:

p-Oxy-m-aminophenylarsonic acid.....	79.88
p-Acetylaminophenylarsonic acid.....	86.53
p-Aminophenylarsonic acid.....	86.35
Phenylglycine-p-arsonic acid.....	82.70

Hence even the six-hour period is too long to bring out marked differences, as four-fifths and more of the arsenic has been excreted by the kidney in this short period. It was impracticable

TABLE 2

Relation between trypanocidal action (effectiveness), toxicity and excretion

SODIUM SALT OF	M. E. D.—1/100 As EQUIVALENT SOLU- TION PER KILO	M. L. D.—1/100 As EQUIVALENT SOLU- TION PER KILO	AVERAGE RECOVERED As IN URINE 6 HOUR PERIOD	AVERAGE RECOVERED As IN URINE 18 HOUR PERIOD	AVERAGE RECOVERED As IN URINE 24 HOUR PERIOD	AVERAGE RECOVERED As IN FECES 24 HOUR PERIOD	AVERAGE TOTAL RE- COVERED As IN URINE AND FECES 24 HOUR PERIOD
	cc.	cc.	per cent	per cent	per cent	per cent	per cent
$R \cdot As = O$							
p-Oxy-m-amino-phenylar- senoxide.....	0.75	10.00	4.24	7.91	13.32	6.65	19.00
Methyl arsenious oxide...	3.75	3.75	4.17	5.68	9.02	1.92	10.94
Arsenious acid.....	7.00	7.00	12.77	0.69	13.46	4.73	19.80
$R \cdot As = As \cdot R$							
Silver arsphenamine.....	0.75	41.00	16.09	5.39	21.48	15.56	37.83
Arsphenamine.....	3.00	50.00	6.05	5.16	11.21	82.35	93.56
Neoarsphenamine.....	3.40	50.00	34.48	19.71	52.00	33.35	87.66
$R \cdot As \begin{matrix} / OH \\ = O \\ \backslash OH \end{matrix}$							
Arsenic acid.....	37.50	50.00	45.66	5.35	51.01	2.00	53.05
p-Oxy-m-amino-phenyl arsonic acid.....	37.50	225.00	79.88	8.79	88.67	4.84	93.66
p-Acetyl-amino-phenyl arsonic acid.....	37.50	750.00	86.53	4.36	90.89	5.10	95.98
p-Amino-phenyl arsonic acid.....	37.50	150.00	86.35	8.62	93.23	5.64	97.97
Phenylglycine-p-arsonic acid.....	375.00	750.00	82.70	14.33	95.80	3.15	98.00

to select still shorter periods however, as the amount of urine secreted would have been so small that the error in its collection would have yielded unreliable figures. In fact it occurred a few times that no urine could be obtained during the six-hour period, therefore requiring repetition of the experiment. The same

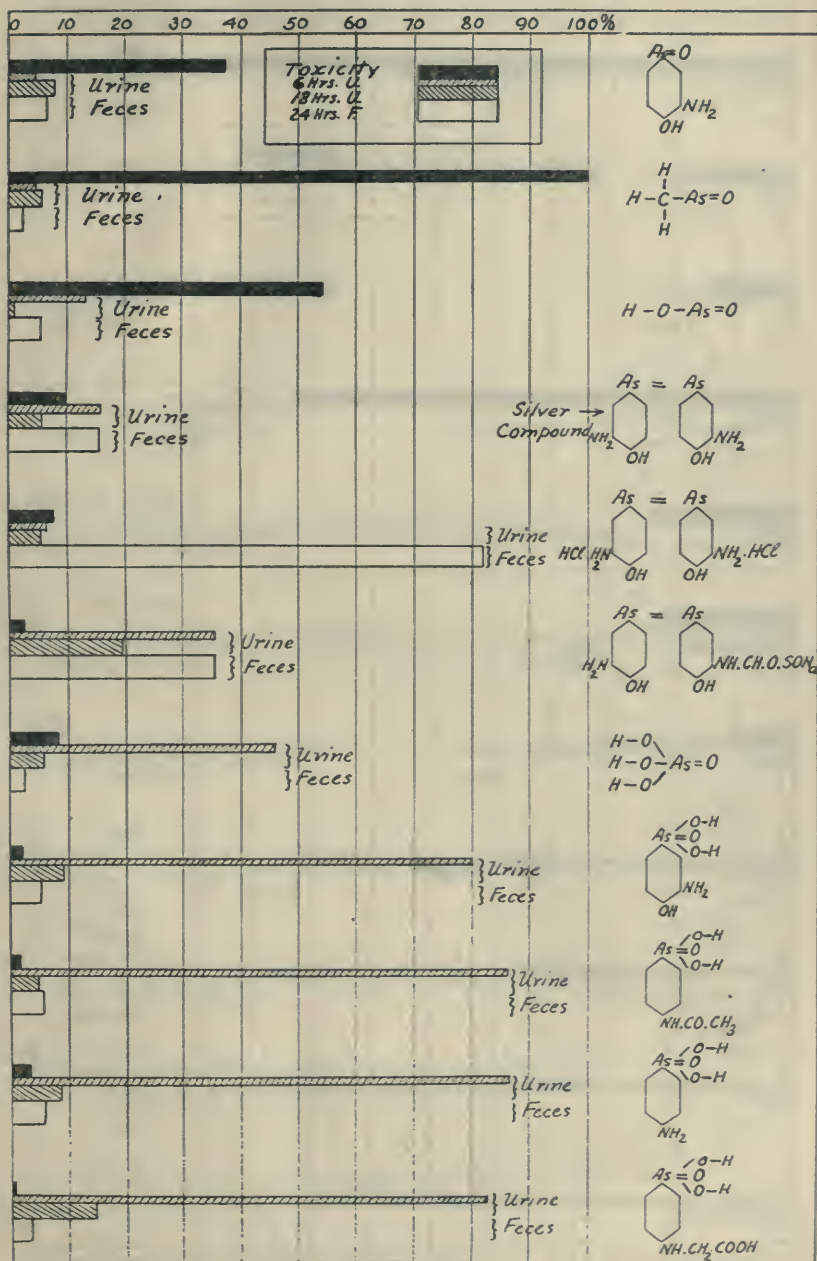


CHART 1

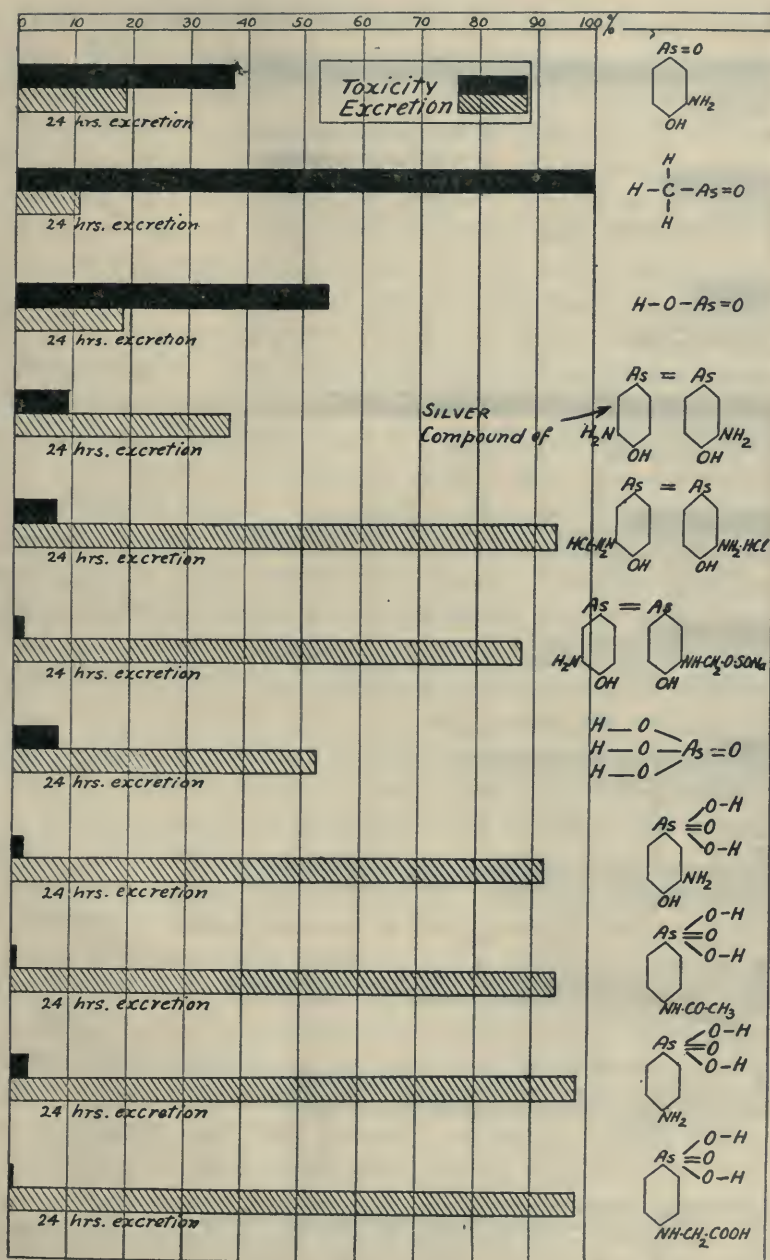


CHART 3

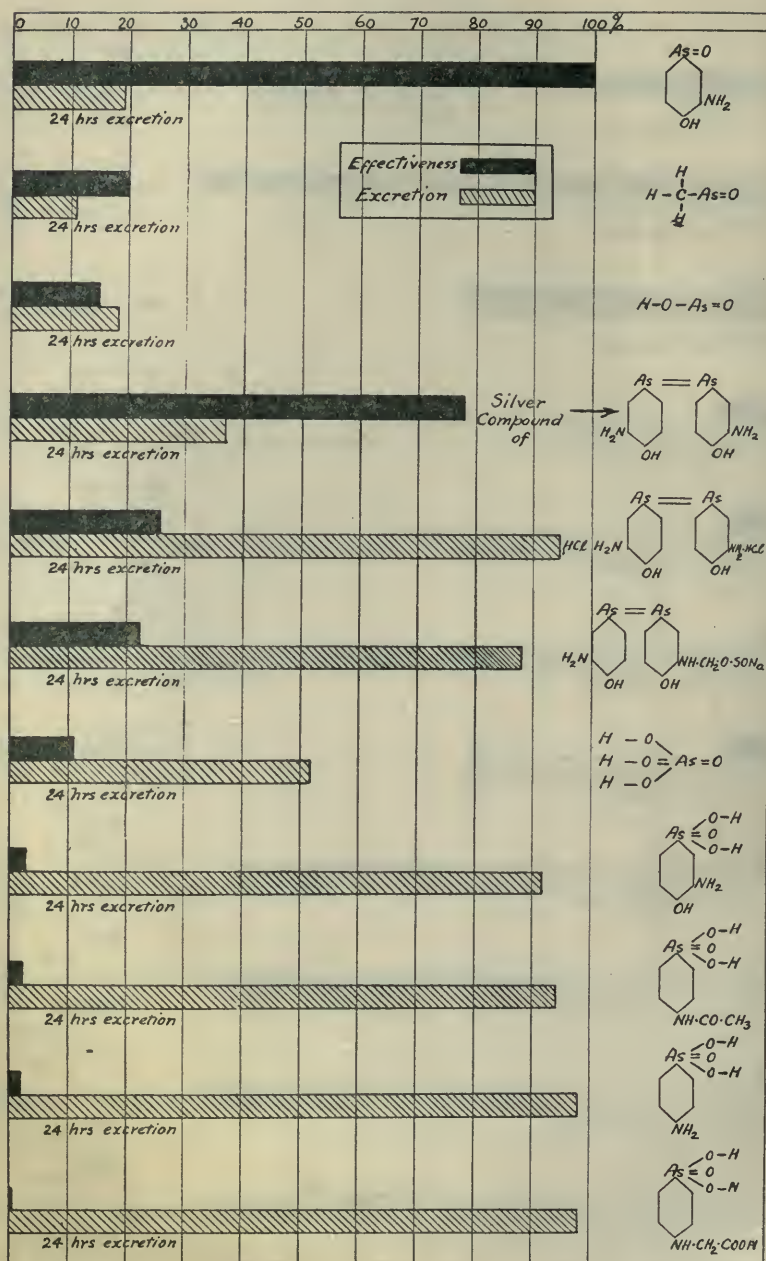


CHART 4

objection holds true for the excretion in the feces. Work on larger animals would undoubtedly have brought out more marked variations. The variations found, however, are quite significant in themselves. Among the trivalent compounds only neoarsphenamine shows a considerable rate of urinary excretion, i.e., 52 per cent in twenty-four hours. The other compounds of these two groups range between 9.02 and 21.48 per cent. Passing to the pentavalent group, there is, as previously stated, a marked increase in rate of urinary excretion, amounting to from 51 to 95.8 per cent in twenty-four hours. The excretion of arsenic into the intestinal tract within twenty-four hours is almost negligible with drugs of the trivalent arsenoxide group and the pentavalent arsenicals; it is, however, considerable with silver arsphenamine (15.56 per cent), and neoarsphenamine (33.35 per cent); and is very great in the case of arsphenamine where 82 per cent of the injected arsenic is excreted into the intestinal tract within twenty-four hours. Different arsenicals evidently leave the body in very different proportions through the kidney and intestinal tract. As a general rule the kidney eliminates the bulk of the arsenic of the pentavalent arsenicals and to a lesser extent that of neoarsphenamine, whereas the arsenic of the arsenobenzene derivatives passes into the intestinal tract in considerable amounts, and in the case of arsphenamine to the greatest extent. The relative ratio of urinary to intestinal excretion can be calculated easily from the data in table 2.

Examination of the rate of excretion, toxicity and effectiveness of the three representatives of trivalent oxides fails to reveal a relation between rate of excretion on the one hand and toxicity and effectiveness on the other; or otherwise expressed, the rate of excretion is not the inverse ratio of toxicity and effectiveness. This lack of correlation is probably due to differences in the physical properties, particularly solubility of these compounds. Methylarsenious oxide, for instance, is lipoid soluble, whereas the other two compounds are not. Lipoid solubility may favor the toxic action by changing the distribution of the drug in such a way as to carry and accumulate the drug in cells of vital importance to the animal. The toxic action upon these cells

might lead to death, whereas a drug which is not lipoid soluble might never reach these same cells in sufficient concentration to produce the same degree of toxic effect. With silver arspenamine, arspenamine and neoarsphenamine an increase in rate of excretion seems to decrease both toxicity and trypanocidal action. In case of silver arspenamine this relation is perhaps not sufficiently emphasized as the toxicity and trypanocidal action of this compound was expressed in terms of arsenic instead of arsenic plus silver. Part of the toxicity and trypanocidal action of silver arspenamine is unquestionably due to the silver component.

The pentavalent arsenicals show the greatest rate of urinary excretion and also the lowest parasitocidal action (charts 2 and 4). This rapid elimination by the kidney accounts for the low effectiveness on the parasites, as these drugs have no direct toxic action, but must be first reduced to the trivalent oxide type. From 80 to 90 per cent of the arsenic of the organic pentavalent compounds is eliminated with the urine within the first six hours following the injection, probably in the form of pentavalent arsenic; the remainder of the drug would obviously not be sufficient, even on the assumption that it be completely reduced to the active trivalent modification, to constitute a minimum effective dose of the corresponding trivalent oxide. The arsenic of arsenic acid shows a slower rate of urinary excretion and this drug is therefore more toxic for the parasites than the other pentavalent arsenicals. A similar relation is shown to exist between the rate of urinary excretion and toxicity for the rat.

Comparison of the rate of excretion of arsenious and arsenic acid furnishes a plausible explanation of the difference of the toxicity of these compounds in the higher animals. The toxicity of arsenite for rats is about seven times greater than that of arsenate. Arsenate is reduced in the body to the directly toxic arsenite; this reduction requires necessarily some time, during which some of the arsenic is excreted. From the figures in table 2 it will be seen that the rate of excretion of the arsenic of arsenate is two-and-a-half times greater than that of arsenite,

a difference which probably accounts for the difference in toxicity of the two compounds in the higher animals.

Summing up the subject so far discussed, we may say that the rate of excretion exerts a potent influence upon the toxicity and parasiticial action of the various arsenicals. The question arises: What is the cause of these differences in the rate and path

TABLE 3
Comparison of rate of disappearance of arsenic from blood stream

SODIUM SALT OF	NUM- BER OF ANI- MALS USED	ARSENIC AS FOUND IN MICROMILLIGRAMS IN 10 CC. OF SERUM							
		Time killed after injection, in minutes							
		15	30	45	60	75	90	105	120
R·As = O									
p-Oxy-m-aminophenyl- arsenoxide.....	13	107.0	71.0	62.5	87.5	92.9	70.0	90.5	33.3
Methyl arsenious oxide.....	5	50.0	47.5	37.5	48.7	68.9			
Arsenious acid.....	5	25.0	50.0	20.0	8.0	6.0			
Phenylarsenoxide.....	5	59.3	143.8	98.0	87.5	71.4			
	5	58.0	39.0	37.0	34.0	14.0			
R·As = As·R									
Silver arsphenamine.....	5	122.5	107.5	92.9	115.0	97.5			
Arsphenamine.....	8	76.0	105.0	98.6	92.6	49.6	49.6	29.6	60.6
Neoarsphenamine.....	8	260.0	192.5	190.0	152.0	90.5	115.0	87.5	70.0
R·As $\begin{matrix} \diagup \text{OH} \\ = \text{O} \\ \diagdown \text{OH} \end{matrix}$									
Arsenic acid.....	5	117.5	109.0	108.0	95.8	16.2			
p-Oxy-m-aminophenylarsonic acid.....	5	121.4	62.5	52.0	21.0	7.7			
p-Acetyl-amino-phenyl-arsonic acid.....	5	80.0	50.0	41.0	20.0	9.0			
p-Aminophenyl-arsonic acid....	5	88.0	78.0	62.0	38.0	31.0			
Phenylglycine-p-arsonic acid...	5	89.0	76.3	72.5	71.4	25.0			

of excretion of the various arsenicals? This question, we believe to be connected with the fundamental laws governing chemotherapy. The data presented on the excretion appear to us to indicate that the chemical constitution of an arsenical determines its retention by the tissues of the host and by the parasites in so far as chemical constitution determines the physical properties. Solubility, molecular weight, surface tension, electric charge and

dissociation are probably very important factors having a bearing on the distribution of a given arsenical in the body, the path and rate of excretion, the toxicity for tissues of the host, and the parasites. Information relating to the physical properties of the compounds used in this investigation is, however, very meager.

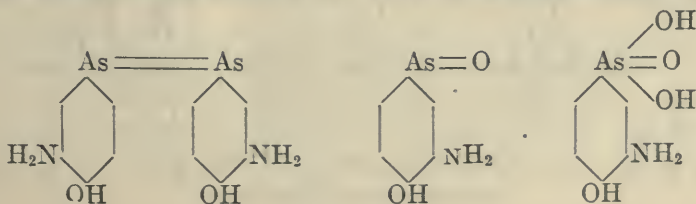
A brief exposition of the factors which may enter into play, however, is not out of place. It is probably not far from the truth to state that the urinary excretion is a fairly good index of the diffusibility of these compounds, the kidney being an organ which eliminates these compounds according to their rate of diffusion in the body. This diffusion appears to be dependent to a considerable extent upon the reaction of the compounds. Passing from the amphoteric substances of the trivalent arsenicals which show a low rate of urinary excretion and presumably low rate of diffusion to the more acid pentavalent compounds we find the rate of urinary excretion and presumably also the rate of diffusion enormously increased. Most of the increase in acidity of the pentavalent compounds can be attributed to the marked acid properties of the pentavalent in contrast to the trivalent arsenic. The introduction of acid groups into the organic radical undoubtedly also plays a part in this change. For instance arsphenamine is amphoteric, is precipitated at the neutral point, and shows a very low rate of urinary excretion (11 per cent in twenty-four hours), whereas neoarsphenamine is more acid in nature on account of the presence of the sulfoxylate group, does not precipitate at pH 7, and is more rapidly excreted with the urine (52 per cent in twenty-four hours). It should be emphasized that probably none or only a very small part of arsphenamine is excreted as such in the urine, as Sieburg (1917) could only isolate pentavalent organic arsenicals and some arsenite from the urine of patients who had received intravenous injections of arsphenamine. The arsenic actually found in the urine therefore does not represent arsphenamine but its oxidation products. The greater diffusibility of the pentavalent organic arsenicals as compared with the trivalent compounds also explains the fact that the former (atoxyl, arsacetin) easily produce symptoms which can be referred to a toxic action upon the central nervous system, whereas arsphenamine has been shown both in

the clinic and in the laboratory to be devoid of such side actions. Permeability of the nervous system and urinary excretion is evidently closely related to the chemical constitution of arsenicals, a view which has lead to further work in the elaboration of drugs for the treatment of neurosyphilis (see Voegtlin and M. I. Smith, 1920).

The data illustrated by chart 5 furthermore add evidence which supports our view of the relation between chemical constitution, rate of urinary excretion and pharmacological action. It will be seen from this chart that the blood serum fifteen minutes after the injection of these drugs contains only a small portion of the arsenic which was injected. Seventy-five minutes after the injection the serum contains only a trace of the arsenic of the pentavalent compounds and considerably more of the trivalent. Phenylarsenious oxide and methylarsenious oxide seem to be exceptions, as they disappear very rapidly from the blood. The latter compound on account of its lipoid solubility is probably removed by tissues rich in lipoids. In general it may be said that the arsenicals leave the blood soon after an intravenous injection. In the case of the trivalent compounds this is probably brought about by fixation to the tissues in a more or less firm combination. The pentavalent compounds, however, probably penetrate the tissues by diffusion without firm fixation, as otherwise the rapid urinary excretion could not be accounted for.

Physical properties are probably also responsible for the different distribution of these drugs in the body and the differences in their paths of excretion. An interesting illustration of this kind is found in arspenamine and its oxidation products:

p-oxy-m-aminophenylarsenoxide and p-oxy-m-aminophenylarsonic acid



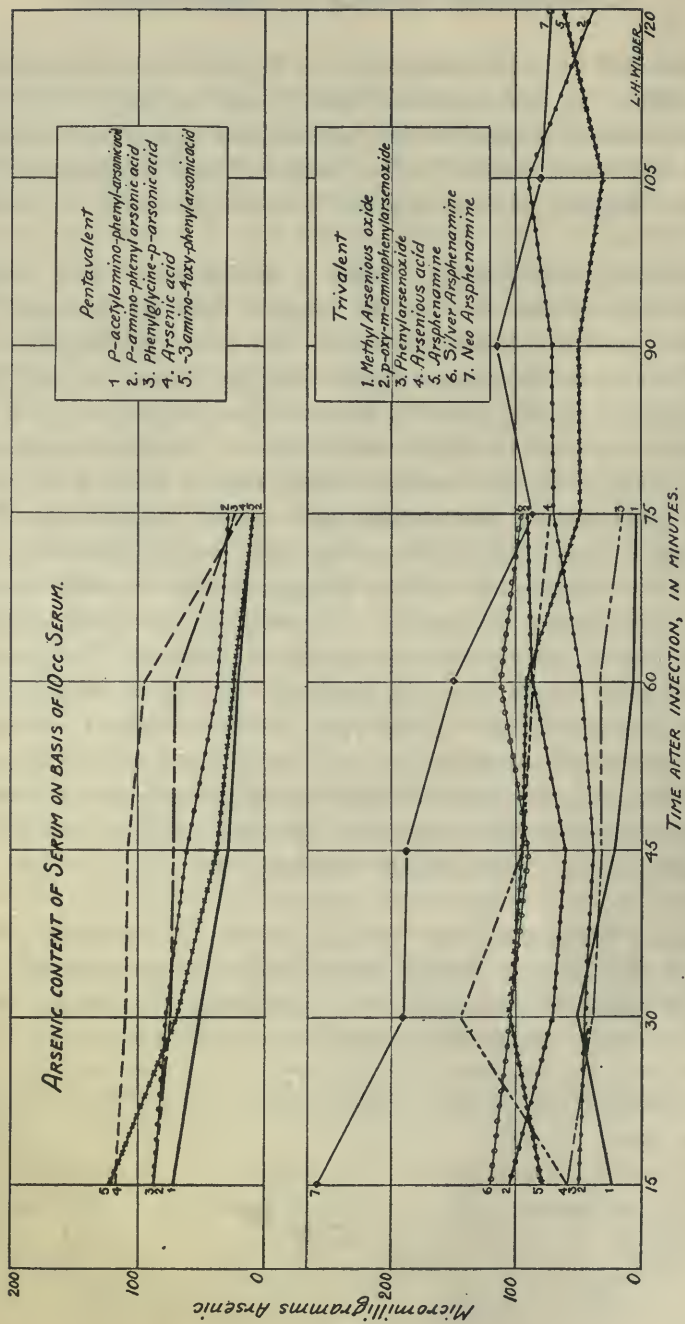


CHART 5

The total twenty-four-hour excretion of these three compounds is 93, 19, and 94 per cent respectively. The arsenic of arspenamine and that of the pentavalent acid compound is therefore almost completely eliminated within twenty-four hours, in contrast to only one-fifth that of "arsenoxide." Moreover, attention is called to the fact that arspenamine is largely excreted with the feces, whereas the arsenic of p-oxy-m-aminophenylarsonic acid appears mostly in the urine. Arspenamine is an amphoteric substance which is probably precipitated soon after injection on account of its insolubility at pH 7.3. Part of this precipitate is caught by the phagocytic cells of the liver, this being followed by the oxidation of the drug within the liver and the subsequent elimination of its oxidation products with the bile. That arspenamine has a special affinity for the liver was shown by Hooper, Kolls and Wright (1921) by histological examination of the liver. Proof for the partial excretion of the arsenic of arspenamine by the liver will be furnished in the next paper of this series. The greater rate of excretion of the arsenic of arspenamine than that of the corresponding oxide is probably due to a difference in the distribution in the body of these two drugs and may account for the therapeutic value of arspenamine. We would have to regard arspenamine as a drug, though not directly toxic to the parasites nor the tissues of the host, which is gradually converted by oxidation into the active "arsenoxide," and on account of its peculiar distribution which favors the liver, is readily excreted mostly in the pentavalent form by the kidney and more so by the liver. That the distribution of the arspenamine in the body must be different from that of "arsenoxide" follows from the fact that the latter is excreted ever so much slower than the former, otherwise the rate of excretion of the arsenic of arspenamine should be less than that of "arsenoxide."

Temporary retention of the arsenic is unquestionably an essential requirement for good chemotherapeutic action, but the optimum is evidently reached in compounds of the arsenobenzene type with a rate of excretion falling between that of the

pentavalent arsenicals and the trivalent directly toxic oxides. This optimum of rate of excretion insures sufficient time for oxidation into the active modification, and on account of favorable distribution, relatively rapid elimination of the arsenic, the latter having caused the death of the parasites, but having not had sufficient time to injure the tissues of the host. The reason for this difference in the time factor of the toxic action on the parasites on the one hand and on the tissues of the host on the other, which makes chemotherapeutic action possible, will be discussed in another paper.

CONCLUSIONS

1. A considerable variation is found in the rate of excretion of arsenic, following the intravenous injection of an arsenical, between different individuals of the same species. This variation is considered as the chief factor responsible for the variation in toxicity and parasitocidal action.

2. Different arsenicals are excreted from the body at a different rate, the directly toxic trivalent oxide compounds showing the slowest rate, the pentavalent arsenicals the fastest, and the arsenobenzene compounds occupying an intermediate position.

3. The ratio between urinary and fecal excretion of the arsenic of different arsenicals shows great variations.

4. The greater part of the arsenic leaves the blood plasma soon after the intravenous injection of the drugs.

5. This research reveals an important relation between rate of excretion on the one hand, and toxicity and parasitocidal action on the other. This relationship appears to depend upon the differences in physical properties of the arsenicals studied. It is pointed out that changes in the chemical constitution affect the physical properties, which in turn determine the rate of diffusion and distribution in the body and the path and rate of excretion of the arsenic, thus governing the toxicity for the host and the therapeutic action.

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THE MECHANISM OF THE STRAUB BIOLOGIC TEST FOR MORPHINE

W. J. R. HEINEKAMP

*From the Laboratory of Pharmacology, University of Illinois, College of Medicine,
Chicago*

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In 1911 Straub (1) published a biologic test for morphine which he thought was specific. Hermann (2), in 1912, described the test stating that the spine assumes a lordotic curvature, the hind legs become slightly spastic and the tail arches in an S-shape over the back. He extended Straub's work and found that opium and all its alkaloids effected the reaction. Papaverine, narcotine, narceine, thebaine, and dionine, however, gave irregular results and only with large doses. Codeine, apomorphine, and heroine must be differentiated in forensic medicine, heroine producing equal or stronger reactions than morphine; but since heroine can be excluded in most cases by the history, and codeine and apomorphine were only effected in ten times the dose morphine was, the test may be taken as characteristic for morphine. He also stated that nicotine produced a similar reaction, but that the hind legs were paralyzed so that the animal could not move. He found strychnine and atropine inactive, but adds "with testing more alkaloids may be found which will produce a similar action on white mice." He also found that extracts of blood, liver, brain, stomach, and intestines from warm blooded animals which had been given morphine gave the test when injected into white mice.

Rübsamen (3), in 1908, while investigating the habituation to morphine noted that white rats injected with morphine held their tails up in a particularly stiff manner due to tonic contraction of the tail muscle. He gave no further explanation. Rassers (4), in 1915, stated that the test was not specific for opium

alkaloids since cocaine, caffeine, diuretine, camphor, tetanus toxin, and potassium cyanate also elicited it; hence it is to be used only as a supplementary test for morphine. Jensen and Rumry (5), working in this laboratory in 1918, confirmed Herrmann's statement that nicotine caused the phenomenon, but do not mention total paralysis of the hind legs was effected, although they do state that the reaction produced by nicotine differed from that caused by morphine in that the animal tended to be quiet.

v. Leersum (6) in 1919, stated that the reaction is the result of vesical and anal tenesmus, the site of the action being in the medulla, the reflex traveling by way of the pelvic nerves, since the phenomenon did not occur when the pelvic nerves were cut. The vesical and anal spasm he thought was of spinal origin. Macht (7), in 1920, stated that the morphine injected into the white mouse or rat produced a spasmodic contraction of the bladder and sphincters. Since he worked with isolated tissue and found that while the phenanthrene portion of the morphine nuclei was without effect on plain muscle, piperidine acted as a powerful stimulant to smooth muscle increasing the rate and strength of its contractions and also its tonicity. He, therefore, concludes that Straub's phenomenon is due to the peripheral effect of the piperidine portion of the morphine molecule. Suitable doses of piperidine hydrochloride when injected into a mouse or rat produces a condition resembling Straub's phenomenon.

The present series of experiments indicate that the phenomenon is not a result of vesical or anal spasm whether of peripheral as stated by Macht or spinal origin, as stated by v. Leersum, but that it is due to direct stimulation of the cord; and that it is not a specific test for morphine since it is produced by other drugs which stimulate the spinal cord.

The following series were performed on male white mice.

Series I. Three white mice

Protocol. November 14, 1921. Mouse anesthetized with ether. Circular incision made about sphincter ani. Large bowel dissected

free and four centimeters of large gut removed after tying off. Skin approximated following operation.

<i>Time</i>	<i>Remarks</i>
9:25	Operation started
9:31	Rectum removed
9:32	1 mg. morphine sulphate subcutaneously
9:35	Mouse about cage
9:45	Tail slightly up. Back hunched
9:47	Test strongly positive

Post mortem: Abdomen opened and explored through median line incision. Free ligated end of gut found. No evidence of lower end of bowel.

Series II. Three white mice

Protocol: November 22, 1921. Mouse anesthetized with ether. Incision in skin made encircling sphincter ani and penis. Urethra, urinary bladder and rectum removed. Skin approximated following operation.

<i>Time</i>	<i>Remarks</i>
1:35	Operation started
1:42	Rectum and bladder removed
1:43	1 mgm. morphine sulphate given subcutaneously
1:47	Test positive
1:50	Test strongly positive

Post mortem: Abdomen opened and explored through median line incision. No evidence of urinary bladder and lower bowel. Free end of ureters found.

Series III

Mouse I

<i>Time</i>	<i>Remarks</i>
9:01	0.65 mgm. morphine sulphate given subcutaneously
9:13	Test strongly positive

Mouse II

9:02	0.65 mgm. morphine sulphate given subcutaneously
9:15	Test strongly positive

Mouse III

9:52	0.5 mgm. morphine sulphate oxidized with concentrated HNO_3 and neutralized to phenolphthalein with NaOH , given subcutaneously
10:04	Test strongly positive

Mouse IV

9:54 0.5 mgm. morphine sulphate (oxidized) given subcutaneously
10:02 Test strongly positive

Series IV

Mouse I

<i>Time</i>	<i>Remarks</i>
2:35	3 mgm. cocaine hydrochloride given subcutaneously
3:02	Test strongly positive. Mouse died in convulsions. Dose too large

Mouse II

2:36 5 mgm. caffeine sulphate subcutaneously
2:50 Test positive
3:00 Test strongly positive

Mouse III

2:37 0.2 mgm. nicotine subcutaneously
2:38 Test strongly positive

Mouse IV

2:41 20 mgm. camphor in oil
2:47 Test strongly positive. Dosage too large. Test strongest during convulsions, but persisted afterward

Mouse V

2:43 1 mgm. codeine phosphate subcutaneously
3:05 Test strongly positive

Mouse VI

2:44 0.05 mgm. strychnine sulphate
3:12 0.025 mgm. strychnine sulphate. Tendency to hold tail up when moving about
3:26 1 mgm. morphine sulphate
3:29 Test positive

Mouse VII

1:58 0.15 mgm. strychnine sulphate
2:03 Spasms. Death. During convulsions tail arched over back

Series V

Mouse I

<i>Time</i>	<i>Remarks</i>
1:00	2 mgm. morphine by mouth. Negative result

Mouse II

1:03	2 mgm. morphine by mouth. Negative result
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Mouse III

1:04	2 mgm. morphine sulphate by rectum. Negative result
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Mouse IV

1:05	2 mgm. morphine by rectum. Negative result
------	--

Mouse V

1:15	0.15 cc. pituitrin subcutaneously. Negative result
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It is evident from the above experiments that Straub's phenomenon is not due to vesical or anal spasm of spinal origin and initiated by morphine as stated by v. Leersum, nor to the direct action of the piperidine portion of the morphine molecule on the vesicle and anal sphincters as indicated by Macht. Both of these theories are disproved by the experiments of series I and II in which the bladder and rectum were removed.

Although v. Leersum states that after section of the pelvic nerves no reaction is obtained, I have found that after complete removal of the sphincter ani, rectum, penis, and urinary bladder an operation which necessitates section of the pelvic nerves, at least the ones supplying these organs, morphine still produces a reaction. Hence the phenomenon cannot be due to vesicle or anal spasm nor to any reflex that may travel over the pelvic nerves.

Although piperidine hydrochloride may increase the tone, rate and strength of contractions of smooth muscle as stated by Macht, it does not necessarily follow since morphine does cause a spasm of the vesicle and anal sphincters that this is the cause of the phenomenon. Persistence of the reaction after removal of these sphincters proves that any local action which piperidine

or morphine may exert on them is not the cause of the test. With both v. Leersum's and Macht's explanation it must be assumed that the impulse spreads to the entire cord. This does not normally occur but can be effected by sensitizing the cord with strychnine.

McGuigan and Ross (8), in 1915, found that morphine when introduced intraspinal in the dog produced strychnine-like spasms, which they stated were due to oxidation products of morphine. They found that morphine treated with concentrated nitric acid and then neutralized produced these spasms more quickly than untreated morphine. They state that it is not morphine *per se* but its products of oxidation that produce the stimulating action when morphine is injected into the system the oxidation normally taking place in the body. In series III, I have found that smaller doses of oxidized morphine produced the phenomenon in the mouse in shorter time than did larger doses of untreated morphine. In other work, to be published, I have found also that morphine treated with nitric acid produced a quicker, more powerful and more lasting stimulation of the cardio-inhibitory center of the turtle than does morphine.

Tamura (9), in 1919, confirmed the theory of McGuigan and Ross, and stated that the substance producing the tetanizing effect was 2-nitrosomorphine which could be prepared in a pure state by the action of nitrous acid on morphine. In a later article he states that the excitatory stage of morphine poisoning is due to the oxidation products of the drug.

That the phenomenon is due to direct stimulation of the spinal cord is first suggested by the lordosis, spastic gait and arching of the tail, all of which are due to spastic contraction of the voluntary muscles. Stimulation or irritation of lower motor neurones gives rise to a spastic condition of the muscles.

Since the phenomenon is produced by nicotine, codeine, camphor, tetanus toxin, potassium cyanate, and strychnine drugs which stimulate the spinal cord and by morphine which has been proved to be a cord stimulant and since the phenomenon occurs after removal of the rectum and bladder, it may be stated that Straub's phenomenon is the result of spinal cord stimulation, and that it is not specific for opium alkaloids.

CONCLUSIONS

The Straub biologic test is due to direct stimulation of the cord. It is not specific for morphine, but is indicative of spinal cord stimulation.

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IPECAC IN THE TREATMENT OF BLACKHEAD IN TURKEYS

H. W. GRAYBILL

From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, New Jersey

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The spring of 1921 Wegeforth and Wegeforth (1) reported results on the treatment of infectious enterohepatitis or blackhead in turkeys. During a period of six seasons they experienced the usual difficulties of those undertaking the rearing of this bird. In September, 1919, after their flock of that year had been decimated and when the outbreak was still in progress, there remained 52 young birds out of a flock of 209 hatched. About that time the early researches of Dr. Theobald Smith on the etiology of the disease came to their attention. The presence of *Amoeba meleagridis* in the lesions suggested as a remedy ipecac, a drug which has proved of value in the treatment of amoebic dysentery in man. They pursued the plan of isolating birds as soon as symptoms developed and placing them under treatment. During the first three days 10 drop doses of fluid extract of ipecac were administered per os three times a day, then for three days two 10 drop doses, and for three days longer one 10 drop dose. Of the 52 birds remaining 32 were attacked by the disease. Of these 29 recovered, making a mortality of about 9 per cent. Of five old birds that developed the disease, three recovered and two died.

In two additional experiments, the authors obtained results which led them to believe that the disease was prevented by the use of fluid extract of ipecac and by powdered ipecac.

In view of these experiments it seemed desirable that further tests with ipecac should be made.

EXPERIMENTS WITH IPECAC

During the summer of 1921 a spontaneous outbreak of black-head supplied a number of cases which were used to test out the value of fluid extract of ipecac as a remedy. The flock was running in a small enclosure and as soon as a bird showed symptoms it was transferred to a nearby pen provided for sick turkeys and at once placed under treatment. The age of the birds when they became ill ranged from twenty-nine to sixty-two days. The doses were usually diluted with a somewhat greater quantity of water. The course of the treatment was adjusted as far as possible to the condition of the bird. Sometimes treatment was omitted for a time because the bird was considered too sick, at other times treatment appeared unnecessary on account of a marked improvement in condition.

In the accompanying table the dosage and results of treatment are given. Nineteen turkeys were treated. Of these, 9 died of blackhead and the rest recovered making a mortality of nearly 50 per cent.

At the time the first turkeys became available for treatment no tests for determining dosage had been made. A few tests were then made on normal turkeys, and later the two controls (no. 20 and no. 21) shown in the table were maintained.

The tests on normal turkeys were on birds twenty-nine to thirty-four days old.

The results were as follows: ten drops a day for two days in 5 drop doses did not prove toxic. Ten drop doses a day for four days proved slightly toxic. Two 10-drop doses for one day and one on the following day proved somewhat toxic. Two 8-drop doses for one day and one 8-drop dose the following day proved somewhat toxic. Two 10-drop doses for one day proved slightly toxic.

The determination of toxicity was based on the presence of symptoms of illness, such as not being normally active, or drooping.

From these results it will be noted by referring to the table that some of the birds received toxic doses. It will therefore be well to consider individually those turkeys that died.

NUMBER OF TURKEY	AGE	DATES OF TREATMENT AND NUMBER OF DROPS GIVEN*																															REMARKS
		July																															
		August																															
	days	1	2	3	4	5	6	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	29	30	1	2	3	4			
1	36	10	10	10	10																										Died July 11		
2	29	10																													Recovered		
3	36	5	5	10	10																										Recovered		
4	36	5	5								10																				Died July 14		
5	36	10	10	10	10																										Died July 22		
6	36	10	10	10	10																										Recovered		
7	39	5	5	10	10																										Died July 13		
8	40	5	5					10	10																						Died July 16		
9	39					10	10																								Died July 22		
10	46							10	10																						Died July 15		
11	41																														Recovered		
12	41																														Recovered		
13	41																														Recovered		
14	44																														Died July 28		
15	48																														Died August 7		
16	53																														Recovered		
17	53																														Recovered		
18	53																														Recovered		
19	62																														Recovered		
20	43																														No toxic effects		
(Control)																																No toxic effects	
21	50																															No toxic effects	
(Control)																																No toxic effects	

* Two figures on same date indicate two doses were given.

Turkey 1 on the fourth day after treatment began appeared normal indicating that there was no important toxic effects at that time. On the fifth and sixth days it was not quite normally active. Treatment was then discontinued. It grew worse and died five days later.

Turkey 4 had improved on the fourth day so that it was normally active. On the fifth and sixth days it was not quite normally active. Then followed a period of six days when treatment was discontinued. It improved during the first half of this period and appeared normal. Then it was not normally active again and died, following another treatment, at the end of fourteen days.

Turkey 5 likewise showed improvement and appeared normal by the fourth day. On the fifth and sixth days it was not quite normal. During an interval of six days when treatment was suspended it first showed improvement to normal and then showed symptoms again. On the thirteenth day it was decidedly sick and treatment was begun again, the dose being decreased after two days. The last course of treatment could hardly be regarded as toxic. It died twenty-two days after treatment was begun.

Turkey 6 received practically the same course of treatment and recovered.

The dosage for *turkeys 7 and 8* cannot be regarded as toxic according to tests made. Treatment of no. 7 was suspended on account of death, and of no. 8 was discontinued for a time on account of its improved condition.

Turkey 9 showed improvement on the sixth to eighth day after treatment began, then became worse, and died four days later, one day after last treatment.

Turkey 10 received 10 drops on four days. In the tests made on normal turkeys, four doses were found only slightly toxic in a considerably younger turkey.

In the two remaining turkeys that died (*no. 14 and no. 15*) the treatment was sufficiently controlled by turkeys no. 20 and no. 21.

From the above survey of the treatment and condition of the birds, it does not seem probable that the treatment could have been injurious to any considerable degree.

The lesions found in the birds that died presented the usual appearance found in untreated birds. In six the liver and both ceca were involved, in two the liver and one cecum, and in the

remaining one the liver was normal but both ceca were involved. The case of turkey 9 was complicated by aspergillosis.

DISCUSSION AND CONCLUSIONS

The absence of a control group of untreated diseased turkeys renders it impossible to indicate how the degree of mortality in the treated turkeys stands to that which would have occurred in the absence of treatment. It should be stated that the mortality was less than usually occurs in young birds. However, our results do not confirm those of Wegeforth and Wegeforth who used turkeys considerably older. It is well known that the resistance of birds to manifest disease presumably as a result of infection increases with age and it is possible that such resistance played an important part in the results obtained by the authors referred to. A mortality of approximately 50 per cent, such as we obtained, indicates that if ipecac has any value in the treatment of blackhead it cannot in all probability be very great.

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EFFECT OF ADRENALIN AND EXTRACTS OF PANCREAS AND LIVER ON BLOOD DEXTROSE

ELLISON L. ROSS AND LLOYD H. DAVIS

Department of Physiology and Pharmacology, Northwestern University Medical School

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Changes in the suprarenal secretion are associated with changes in the concentration of dextrose in the blood. Some consider an increase in the amount of adrenalin in the blood is all that is necessary to produce a hyperglycemia.

Extirpation of the pancreas results in a marked hyperglycemia and glycosuria. Allen (1) considers that there is sufficient evidence to consider diabetes in many cases the result of a diseased pancreas. The view that the pancreas produces an internal secretion which is an inhibitor to glycogenolysis is not a new one.

Our experiments were made on the hypothesis that the amount of dextrose in the blood is governed by two substances: adrenalin, which tends to increase glycemia, and the internal secretion of the pancreas, which decreases glycemia. The influence of these two factors is most probably exerted in the lysis or synthesis of glycogen. We sought, therefore, to determine the effect of intravenous injections of adrenalin and of the extract of pancreas upon the amount of sugar in the blood.

EXPERIMENTAL WORK

Dogs were used in this work. They were bled from the jugular vein before injection with the various preparations, fifteen minutes after injection, and one hour following injection. The amount of dextrose in the blood was determined by

the method of Benedict (2). The results in the tables are the averages of triplicate determinations.

We introduced into the leg veins of the first group of dogs 0.25 cc. of adrenalin per kilogram of body weight. Sollman (3) quotes Lesage as giving this quantity of adrenalin as the minimal fatal dose of the drug. In none of our animals did we see any severe reaction.

The second group of dogs was given intravenously 1 cc. of pancreatic extract per kilogram of dog, prepared within two hours before the injection. There were no visible reactions from these injections. The extract was made by grinding in a mortar with ground glass 25 grams of pancreas. This was added to 100 cc. of normal salt solution and mixed. It was stirred at intervals and at the end of half an hour was filtered through gauze. The filtrate was used for injection.

A third group of dogs was given the same dose of pancreatic extract which was immediately followed by the usual dose of adrenalin.

A fourth group of dogs was given the same preparations as the second except that the preparations were incubated together for thirty minutes previous to the injection. In two of the animals there was some reaction shown by temporary prostration followed by vomiting.

The results of the injections of adrenalin and pancreas are given in table 1.

Preparations of liver were made in exactly the same way as those from pancreas. The same dosage was used. Three groups of animals were employed and the injections were analogous to the earlier ones of adrenalin and pancreas. The third group which was given the incubated mixture gave results of such a nature that it was thought best not to include them in this paper but reserve them for further investigation. The results from the other two groups are given in table 2.

In table 3 are the average glycemias calculated from tables 1 and 2. In table 4 the average glycemias are expressed in per cents of the normal, taking the estimations before injection as normal.

TABLE 1

Blood sugar changes induced by adrenalin and pancreas intravenous injections.

DOG	MATERIALS INJECTED	RESULTS EXPRESSED IN PER CENT DEXTROSE		
		Before	After 15 minutes	After 1 hour
1	Adrenalin.....	0.083	0.150	0.131
2	Adrenalin.....	0.066	0.113	0.098
3	Adrenalin.....	0.090	0.136	0.105
4	Adrenalin.....	0.098	0.163	0.121
5	Adrenalin.....	0.083	0.120	0.094
6	Pancreas.....	0.089	0.088	0.118
7	Pancreas.....	0.090	0.097	0.105
8	Pancreas.....	0.100	0.057	0.080
9	Pancreas.....	0.079	0.061	0.095
10	Pancreas.....	0.097	0.050	0.083
11	Pancreas followed by adrenalin.....	0.095	0.117	0.101
12	Pancreas followed by adrenalin.....	0.104	0.164	0.114
13	Pancreas followed by adrenalin.....	0.100	0.160	0.113
14	Pancreas followed by adrenalin.....	0.081	0.109	0.117
15	Pancreas followed by adrenalin.....	0.097	0.115	0.075
16	Pancreas and adrenalin incubated.....	0.106	0.101	0.116
17	Pancreas and adrenalin incubated.....	0.083	0.099	0.098
18	Pancreas and adrenalin incubated.....	0.109	0.113	0.100
19	Pancreas and adrenalin incubated.....	0.100	0.066	0.117
20	Pancreas and adrenalin incubated.....	0.097	0.076	0.083

TABLE 2

Blood sugar changes induced by adrenalin and liver intravenous injections

DOG	MATERIALS INJECTED	RESULTS EXPRESSED IN PER CENT DEXTROSE		
		Before	After 15 minutes	After 1 hour
21	Liver.....	0.092	0.096	0.109
22	Liver.....	0.100	0.112	0.105
23	Liver.....	0.110	0.103	0.111
24	Liver.....	0.121	0.135	0.151
25	Liver.....	0.100	0.096	0.115
26	Liver followed by adrenalin.....	0.087	0.144	0.116
27	Liver followed by adrenalin.....	0.102	0.157	0.118
28	Liver followed by adrenalin.....	0.100	0.247	0.202
29	Liver followed by adrenalin.....	0.098	0.165	0.144
30	Liver followed by adrenalin.....	0.094	0.144	0.117

TABLE 3

Average blood sugar changes induced by adrenalin and tissue injections

MATERIALS INJECTED	RESULTS EXPRESSED IN PER CENT DEXTROSE		
	Before	After 15 minutes	After 1 hour
Adrenalin.....	0.0840	0.1364	0.1098
Pancreas.....	0.0910	0.0706	0.0962
Pancreas followed by adrenalin.....	0.0954	0.1330	0.1040
Pancreas and adrenalin incubated.....	0.0990	0.0910	0.1028
Liver.....	0.1046	0.1084	0.1182
Liver followed by adrenalin.....	0.0962	0.1714	0.1394

TABLE 4

Average blood sugar changes induced by adrenalin and tissue injections

MATERIALS INJECTED	RESULTS EXPRESSED IN PER CENT NORMAL		
	Before.	After 15 minutes	After 1 hour
Adrenalin.....	100	162	131
Pancreas.....	100	77	105
Pancreas followed by adrenalin.....	100	139	109
Pancreas and adrenalin incubated.....	100	92	104
Liver.....	100	104	113
Liver followed by adrenalin.....	100	178	145

DISCUSSION

The effect of 0.25 cc. of epinephrin (1:1000) per kilogram of animal, injected into the leg vein, brought on a hyperglycemia in every animal. Fifteen minutes after the injection the amount of blood sugar was much greater than at the end of an hour. The average glycemia before injection was 0.840 per cent of dextrose. Fifteen minutes after the injection it was 0.1364 per cent and one hour after it was 0.1098 per cent. There was an increase in fifteen minutes of 62 per cent and in one hour the increase was only 31 per cent above normal.

The fresh pancreatic extract brought on a decrease in the blood sugar in every case except one and in this one the increase was only 0.007 per cent. The group of animals averaged before

injection 0.0910, fifteen minutes after 0.0706 and one hour after injection 0.0962 per cent. There was a decrease in blood sugar of 23 per cent at the end of fifteen minutes and an increase of 5 per cent at the end of one hour.

When the fresh pancreatic extract was injected, followed immediately by adrenalin, an interesting change took place. In each case both for the periods of fifteen minutes and one hour there was an increase except in one observation, that for the hour period for the last animal of the series. The average before injection was 0.0954 per cent while fifteen minutes later it was 0.1330 and one hour after injection it was 0.1040. In fifteen minutes after injection of these two substances the blood dextrose increased 39 per cent and in one hour the increase was only 9 per cent.

When the incubated mixture of pancreas and adrenalin was administered the tendency was toward a reduction in the glycemia at the end of fifteen minutes but at the end of an hour the tendency was upward. The average glycemia before injection was 0.099 per cent, fifteen minutes later 0.091 per cent and at the end of an hour 0.1028 per cent. Expressed in percents of the normal, there was a decrease of 8 per cent during the first period and an increase of 4 per cent in the long period.

The data given in table 4 on the results of injections of adrenalin and pancreas indicate several things as to the mechanics of action of the two preparations. The algebraic sum of the changes, at the end of fifteen minutes, obtained by the injection of adrenalin and pancreas separately, is 39 per cent—exactly the same result as that obtained by injecting the same substances at the same time into the same animals. It is also interesting to note that for the same period the injection of the same substances incubated together before using gave decidedly different results. At the end of an hour the algebraic sum of the changes due to single injections was 36 per cent which differs materially from both of the changes obtained by the combined injections. It is significant, however, that the change brought on by the two preparations injected together was practically unaltered by incubating the mixture before injection. The results on injec-

tions of adrenalin and pancreas suggest that when the two are administered at the same time they act separately and as opposites on the glycemia of the animal during the first fifteen minutes, and that by the end of an hour the pancreatic substance and the adrenalin have reacted with one another in the body the same as if incubated together outside the body. This being the case we should expect that in the normal living body the active pancreatic substance and adrenalin would appear in relatively small quantities at a time and that they would react together, the surplus of the larger quantity exerting its influence—if the pancreatic substance, decreasing glycemia and if adrenalin, increasing glycemia.

In order to determine whether or not this property of pancreatic extract were not the property of an extract of any parenchymatous organ, similar work was done with preparations of liver. The extract of liver alone brought on slight increases, 4 per cent in fifteen minutes and 13 per cent in one hour. There was no characteristic fall in the glycemia from the liver preparation such as occurred from the injection of pancreatic preparation. When both adrenalin and liver preparations were injected at the same time there was present the characteristic increase of adrenalin and added to it that of the liver preparation. Incubating the liver preparation with adrenalin gave a substance that could not be used for comparative purposes. Further work on this phase of the problem will be done by us. It is clear, however, that the properties of our pancreatic extract were not those of an extract of any parenchymatous organ.

Much work has been done with pancreatic extracts by many investigators. Allen (4) quotes their conclusions and describes some of the essentials of their work. Some have obtained changes in the urine due to injections of pancreatic extracts and some have not. Some have used watery extracts of pancreas of indefinite age, some have used glycerin extracts and some have used sterilized preparations. It is a question what sort of preparations would contain "pancreas hormone" and whether it would be resistant to heat or the digestive properties of the enzymes present. We have found that our pancreatic extracts

gave very different results if kept on ice for 24 hours. Either the digestive ferments present destroyed the "hormone" or the digestive products masked its action. Most of these injections were subcutaneous and the tests were on the urine and not the blood. We have been unable to find record of any procedure that is comparable with ours.

Zuelzer (5) and Zuelzer, Dohrn and Marxer (6) prepared the pancreatic "hormone" which neutralized the glycosuric action of adrenalin, decreased the glycosuria of depancreatized dogs and reduced the glycosuria of human diabetics. Zuelzer considered that in normal life there are two influences in the body governing the liberation of dextrose into the blood, one exerted by the pancreatic "hormone" and one by adrenalin.

Langfeldt (7) has reviewed the essential literature up to the present and has presented a theory of glycemia very similar to that of Zuelzer. Langfeldt states that normally glycogenolysis takes place as a result of the influence of the liver diastases modified in their activity by the hydrogen ion concentration, and that the constant formation of glycogen is being carried on by the pancreatic hormone. He explains that the hyperglycemias are either the result of the failure of the pancreas or the result of increased flow of adrenalin in the presence of thyroiodine, increasing the activity of the liver diastases at the normal hydrogen ion concentrations. Langfeldt has introduced factors that Zuelzer did not but the essentials, that the internal secretion of the pancreas and adrenalin balance one another in the regulation of glycemia, is common to both. This mechanism described by Langfeldt harmonizes with the conclusions reached by us (8) and (9) as to the mechanism of "ether hyperglycemia" and "chloroform hyperglycemia." We concluded that the hyperglycemia produced by ether or chloroform anesthesia was due largely to a reduced activity of the pancreas.

The results presented in this paper on the action of pancreatic extract and extract of liver lead us to conclude, with Langfeldt, that the pancreatic hormone reduces glycemia, specifically. Our results presented in this article on the injections of pancreatic extract and adrenalin lead us to agree with Langfeldt that

glycogenolysis is influenced by adrenalin and that the formation of glycogen is influenced by a pancreatic hormone. Our data strongly suggests an addition to the mechanism of hyperglycemia as described by Langfeldt. He says essentially that the two substances acting on the same thing produce opposite changes in the same place at the same time. Our results suggest a different mechanism, in that the two substances react together and that the one in excess exerts its influence to either build or tear down glycogen.

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QUANTITATIVE STUDIES IN CHEMOTHERAPY
VII. EFFECT OF LIGATION OF THE URETERS OR
BILE DUCT UPON THE TOXICITY AND TRYPAN-
OCIDAL ACTION OF ARSENICALS

CARL VOEGTLIN, HELEN A. DYER, AND DOROTHY WRIGHT MILLER

*Division of Pharmacology, Hygienic Laboratory, United States
Public Health Service*

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In the preceding paper of this series (Voegtlin and Thompson, 1922) observations were reported on the relative rate of excretion of the arsenic of different inorganic and organic compounds. These data, when brought into relation with the toxicity and trypanocidal action of the same drugs, justified the assumption that the rate of excretion has a potent influence upon the toxicity and parasitocidal action. It was furthermore pointed out that the chemical constitution determines the retention and distribution of these drugs in the body and secondarily the toxicity and parasitocidal action, in so far as chemical constitution is responsible for the physical properties of the drugs. The data particularly emphasized the relation between chemical constitution and diffusibility. These conclusions were based upon indirect evidence. On account of their importance it was very desirable to furnish direct proof for this hypothesis and to give it the character of a theory. It is obvious that the correctness of our view would yield a more reliable basis for the theory of chemotherapeutic action than the rather speculative chemoreceptor theory of Ehrlich (1900), which it will be agreed, is not well supported by experimental evidence. Ehrlich accepts a purely chemical theory of drug action, which assumes that the drug is fixed by the parasites and the tissue cells by means of certain

chemical groups (sidechains or chemoceptors). The drugs of therapeutic value are those which possess a maximum affinity for the parasitic chemoceptors and no or only a slight affinity for the receptors of the tissues. In contrast to this hypothesis, we have advanced experimental evidence which for the first time seems to indicate that purely physical factors are of importance in chemo-therapeutic action.

The method used for proving that our views are correct is extremely simple. It should be possible to demonstrate, by preventing the removal from the body of easily diffusible and therefore easily excreted arsenicals, that these drugs would become more toxic for the tissues of the host and the parasites. Inasmuch as the kidney eliminates the greater part of the arsenic of some arsenicals, ligation of both ureters presents a feasible method for blocking this path of excretion. With regard to the excretion with the feces the matter is not so simple. It occurred to us, however, that the arsenic appearing in the feces might have been excreted with the bile into the intestinal tract, this being followed by incomplete reabsorption. Should this be so, then ligation of the bile duct would serve our purpose.

EXPERIMENTAL

As in the previous work healthy, nonpregnant albino rats of approximately 120 grams body weight were selected. The ligation of the ureters is a simple operation, particularly on account of the high resistance of rats to bacterial infections. Antiseptic methods are sufficient to avoid secondary infections. As a matter of fact we have not lost any animals among the several hundred operated upon due to this cause. The operating area was treated with tincture of iodine before incision and after closure of the wound. Care must be exercised in the ligation of the ureters so as to prevent all escape of urine after the operation; this is easily accomplished by placing the ligature around both ureters a short distance above their entrance into the bladder. A careful autopsy was made in every case at the end of the experiment for the purpose of seeing whether the obstruction had been

complete, as also for the detection of gross pathological lesions. The ureters are always found dilated with urine, the kidneys edematous and often much enlarged. In control experiments on eight rats (no drug injected) it was shown that this operation, which requires only five minutes, is well tolerated by the animals, the rats appearing normal within less than half an hour after the operation. For three or four days nothing abnormal is observed; the animals die however soon after the fourth day of uremia. For this reason the toxicity of the drugs was taken as the minimum dose which would kill within forty-eight hours. It was thought that choosing such a short period would eliminate as much as possible the toxic effect of the accumulation in the blood of the normal metabolism products which are ordinarily excreted with the urine. Control experiments on the toxicity and trypanocidal action were made with each drug on rats which had been subjected to the same operation with exception of the ligation of the ureters. This seemed necessary in order to avoid the legitimate criticism that the change in toxicity and trypanocidal action observed in the rats with ligated ureters might be due to the after effects of the anesthesia. That the toxicity is slightly raised as a result of the anesthesia per se is shown in the tables.

The obstruction of the bile duct was carried out in the following manner. A portion of the skin just below the sternum was shaved and painted with tincture of iodine. Through a short incision in the linea alba, the upper portion of the duodenum was pulled out of the peritoneal cavity and brought into plain view the bile duct. A double ligature of surgical silk was placed and tied around the duct a short distance above its entrance into the gut, the intestine placed back into the abdominal cavity and the wound sewed up by stitches in the peritoneum and fascia and the skin separately. Rats do not have a gall-bladder, a fact which makes this species especially suited for our purpose. The operation is well tolerated, the animals apparently remaining free of any pathological manifestations for several days with exception of the urine which assumes an intense yellow color, due to bile pigments. Eight control animals with ligated bile

duct but not injected with any drug, survived for seven days, when they were killed by chloroform. The autopsy revealed complete obstruction of the bile duct, which was engorged by stagnant bile. The liver lobules were indistinct and there was present in every case an icterus of various intensity. No other gross changes were found. The toxicity and trypanocidal action was established in rats with ligated bile duct and operated controls without ligation of the duct.

The drugs were always injected intravenously in the form of aqueous solutions of the sodium salts. The doses are expressed, as in our previous work, as cubic centimeters of a $\frac{1}{100}$ arsenic equivalent solution. An hour and a half to two hours always elapsed between the operation and the injection of the drug. For the determination of the trypanocidal action a strain of *Trypanosoma equiperdum* was used which was somewhat less resistant to some of the arsenicals than the strain employed in previous work.

The tables contain the essential data. In the column headed "Time of death" of the toxicity tables, D stands for death and S for survival. The autopsy notes were restricted to those attributable to drug action, whereas the effect of ligation of the ureters upon the kidney and of the bile duct upon the liver were not mentioned, as they are of no interest.

DISCUSSION

Before proceeding to the discussion of the results it should be pointed out that on theoretical grounds complete obstruction of one path of excretion does not necessarily prevent a compensatory excretion through other channels. In fact this seems to occur to a slight extent as will be evident from an examination of the data. The increased toxicity and trypanocidal action actually observed in these experiments therefore represents merely the minimum effect obtainable from blocking of one of the paths of excretion, and also includes the reduction of the toxicity and trypanocidal action which must be attributed to a compensatory excretion through other channels.

Effect of ligation of both ureters. The data in tables 1, 2 and 5 bring out very clearly the fact that the toxicity and trypanocidal action of some, but not all, arsenicals is increased by ligation of both ureters. As a matter of fact the results obtained were anticipated from the data on the rate of excretion of the arsenic of these compounds (Voegtlin and Thompson, 1922). It will be seen that those drugs which are very slowly excreted by the kidney, show the same toxicity and trypanocidal action in animals with ligated ureters as in normal rats. For instance no change is observed in the minimum lethal dose and minimum effective dose of p-oxy-m-aminophenylarsenoxide ("arsenoxide"), sodium arsenite and arsphenamine. The arsenic recovered in the urine within the first twenty-four hours following the injection of these drugs is 13, 13 and 11 per cent respectively. Passing to the next two drugs, namely neoarsphenamine and sodium arsenate, it is seen that ligation of both ureters increases considerably both toxicity and trypanocidal action (2 to $3\frac{1}{2}$ times respectively).

The therapeutic ratio $\frac{\text{M.L.D.}}{\text{M.E.D.}}$, however, remains the same. The

previous work had shown that these arsenicals are excreted with the urine much faster than the first three drugs, i.e., 52 (neoarsphenamine), and 51 (arsenate) per cent respectively in twenty-four hours. The effect of ligation of the ureters upon the toxicity is greatest with the pentavalent organic arsenicals, p-oxy-m-aminophenylarsonic acid (5 times), arsacetin (5 times), atoxyl (30 times) and phenylglycin-p-arsonic acid (7 times). A similar increase is noted in the trypanocidal action of these drugs, namely 50, 37.5 4.5 and 17 times respectively. In conformity with this great increase in toxicity and trypanocidal action we find that the 24 hour excretion of arsenic with the urine is as follows: 88, 90, 93, and 95 per cent respectively. The influence

of ligation of the ureters on the therapeutic ratio $\frac{\text{M.L.D.}}{\text{M.E.D.}}$ is

brought out in table 5 and shows a very large ratio for p-oxy-m-aminophenylarsonic acid (100) and arsacetin (105). The first one of these compounds is an oxidation product of arsphenamine

TABLE 1
Effect of ligation of both ureters on toxicity

URETERS LIGATED			CONTROLS			
Symptoms		Time of death	Autopsy	Symptoms	Time of death	Autopsy
m-Amino-p-oxyphenylarsenious oxide						
cc.		days			days	
1.5	Absent	S 4	Ascites			
2.25	Absent	S 4	Ascites; liver congested			
3.75	Absent	D 4	Kidneys yellowish-white;	Absent	S 4	Negative
5.0	Absent	D 4	liver pale			
7.5		D 1	Lungs edematous. Liver fatty	Convulsions	D 1	Kidneys congested; liver mottled
10.0	Convulsions	D 1	Liver pale	Convulsions		Liver mottled; kidneys congested
15.0				Convulsions	D 1	Liver pale, lobulation indistinct; kidneys congested
Sodium arsenite						
2.25	Droopy, diarrhea	D 4	Ascites			
2.25	Absent	D 3		Absent	S 5	Negative
3.75	Slight tremors	D 3	Slight ascites	Drowsy	S 4	Liver pale
5.0	Drowsy	D 1			D 1	Negative
5.0	Drowsy	D 2	Kidneys "white"			
5.0	Unable to walk	D 2	Kidneys yellowish-white; liver congested			
7.0		D 1			D 1	Negative

Arsphenamine

10.0	Droopy	D 4	Kidneys pale pink	Absent	S 4	Negative
15.0	Droopy	D 4	Ascites	Absent	S 4	Negative
22.5	Absent	D 3	Kidneys edematous; liver mottled			
37.5	Diarrhea, droopy	D 3	Ascites; kidneys pale; liver congested	Diarrhea	S 4	Negative
37.5	Diarrhea	D 2	Ascites; kidneys pale; liver congested	Salivating	D 2	Liver fatty; kidneys pale
50.0	Absent	D 2	Increase in pleural fluid; kidneys mottled		D 1	Liver pale yellow, fatty change
50.0	Diarrhea	D 3	Ascites; excessive pleural fluid; liver and kidneys congested		D 1	Increase in pleural fluid; liver mottled, pale; spleen large, congested
75.0	Tremors	D 2	Increase in pleural fluid; kidneys and liver pale; small intestines hemorrhagic		D 1	Spleen congested. Lungs slightly congested

Neosphenamine

15.0	Slight tremors	S 4	Negative			
22.5	Slight tremors, diarrhea	S 4	Ascites			
37.5	Tremors, ataxia, diarrhea	S 4	Kidneys "white," ascites	None	S 5	Negative
50.0	Tremors, ataxia, diarrhea	S 4	Kidneys "white," large, edematous	None	S 5	Negative
75.0		D 1		None	S 5	Negative
100.0		D 1		Tremors, ataxia, diarrhea	S 5	Kidneys very pale
150.0					D 1	Kidneys and spleen congested

TABLE 1—Continued

URETERS LIGATED							CONTROLS						
DOSE		Symptoms		Time of death		Autopsy		Symptoms		Time of death		Autopsy	
Sodium arsenate													
cc.				days						days			
3.75	Absent			S 4		Kidneys pale pink							
5.0	Absent			D 4		Kidneys pale pink							
7.5	Absent			S 5		Kidneys pale pink							
10.0	Tremors, paralysis			D 2		Liver fatty		Absent		S 2		Negative	
15.0				D 1				Absent		S 4		Negative	
22.5	Paralysis, cyanosis			D 3 hrs.		Lungs congested		Absent		S 4		Negative	
37.5	Paralysis, cyanosis			D 3 hrs.		Lungs congested		Paralysis		D 1		Liver pale	
50.0	Paralysis, cyanosis			D 2 hrs.				Paralysis, cyanosis		D 1		Liver pale; right lung slightly congested	
75.0	Paralysis, cyanosis			D 30 min.				Paralysis		D 45 min.			
p-Oxy-m-aminophenylarsonic acid (sodium salt)													
7.5	Absent			S 5		Negative							
10.0	Slight tremors			S 5		Negative						S 5	
15.0	Severe tremors, ataxia			S 5		Kidneys "white;" ascites		Absent				S 5	
22.5	Severe tremors, ataxia			S 4		Kidneys pale pink; ascites		Absent				S 5	
22.5	Tremors, paralysis, diarrhea			D 2		Negative		Absent				S 5	

37.5	Tremors, ataxia	S 5	Negative	Hyperexcitable	S 5	Negative
37.5	Tremors, ataxia	D 3	Kidneys pale	Tremors, ataxia	S 5	Negative
50.0	Tremors, ataxia	S 5	Negative	Slight tremors;	S 5	Negative
75.0	Tremors, ataxia	D 2	Pleural fluid increased	ataxia		
100.0		D 1	Kidneys pale	Tremors, ataxia	S 5	Negative
150.0				Tremors, ataxia	S 5	Negative
225.0				Tremors, ataxia	S 5	Negative
375.0					D 1	Spleen congested; Kidneys pale
Arsacetin						
7.5	Tremors, ataxia	S 4	Ascites			
10.0	Hyperexcitable	S 4	Kidneys pale pink; spleen congested, large			
15.0	Tremors, ataxia	S 4	Kidneys pale	Absent	S 5	Negative
22.5	Tremors, ataxia, paralysis	D 4	Kidneys "white," intestines hemorrhagic			
37.5	Tremors, ataxia, paralysis	D 4	Kidneys pale			
50.0	Tremors, ataxia, paralysis	D 4	Kidneys mottled; liver congested			
75.0	Tremors, ataxia, paralysis	D 3				
105.0	Tremors, ataxia, paralysis	D 2				
225.0				Tremors, ataxia	S 5	Negative
375.0				Tremors, ataxia	S 5	Negative
500.0				Tremors, ataxia	D 3	Kidneys pale pink
750.0					D 1	Lungs, liver and spleen congested

TABLE 1—Concluded

DOSE	URETERS LIGATED			CONTROLS		
	Symptoms	Time of death	Autopsy	Symptoms	Time of death	Autopsy
AtoxyI						
cc.		days			days	
1.0	Absent	S	Ascites			
1.5	Hyperexcitable	D 4	Ascites; liver congested			
2.25	Hyperexcitable	D 4	Bloody ascites			
3.75	Tremors, diarrhea	S 4	Negative			
5.0	Tremors, ataxia	D 2	Kidneys pale			
7.5	Tremors, ataxia	D 2	Kidneys pale; ascites			
10.0	Tremors, ataxia, paralysis	D 2	Kidneys pale; ascites			
15.0						
22.5	Tremors, ataxia, diarrhea, paralysis	D 3	Kidneys pale; liver congested	Absent	S 5	Negative
				Absent	S 5	Negative
37.5	Tremors, ataxia	D 2		Slight tremors	S 5	Negative
50.0	Tremors, ataxia, diarrhea, paralysis	D 2	Kidney pale, liver congested; intestines slightly hemorrhagic	Slight tremors	S 5	Negative
75.0				Severe ataxia tremors,	D 4	Negative
100.0				Severe ataxia tremors,	D 3	Negative
150.0				Severe ataxia tremors,	D 1	Small intestines slightly hemorrhagic

Phenylglycin-p-arsonic acid (sodium salt)

5.0	Droopy	D 4	Kidneys pale; liver congested			
7.5	Slight tremors	D 4	Kidneys pale pink			
10.0	Tremors, ataxia	D 2	Kidneys pale; ascites			
15.0	Absent	D 3	Kidneys pale			
22.5	Tremors	D 2	Kidneys edematous; ascites			
37.5	Tremors	D 3	Kidneys pale; liver congested			
50.0	Drowsy	D 2	Kidneys "white"			
75.0	Diarrhea, tremors, convulsions	D 2	Kidneys "white," edematous	Absent	S 5	Negative
102.5	Hyperexcitable, tremors, diarrhea	D 2		Absent	S 5	Negative
150.0				Absent	S 5	Negative
225.0				Absent	S 5	Kidneys pale
375.0				Tremors, hyperexcitable, convulsions	D 1	Typical "white" kidney
500.0				Severe tremors, paralysis	D 1	

TABLE 2
Effect of ligation of both ureters on trypanocidal action

DOSE	URETERS LIGATED			CONTROLS		
	Initial count	24 hours	48 hours	Initial count	24 hours	48 hours
m-Amino-p oxyphenylarsenoxide						
cc.						
0.05	116,000	296,000	+++ Killed	98,000	440,000	+++ Killed
0.075	108,000	48,000	+++ Killed	147,000	280,000	+++ Killed
0.1	114,000	16,000	+++ Killed	95,000	22,000	+++ Killed
0.15	146,000	—	— Killed	115,000	14,000	+++ Killed
0.225	160,000	Trace	+ Killed	120,000	Trace	Trace, Killed
0.375	123,000	—	— Killed	170,000	—	— Killed
0.5				166,000	—	— Killed
0.75	170,000	—	— Killed	180,000	—	— Killed
Sodium arsenite						
1.0	184,000	632,000	Dead			
1.0	122,000	320,000	Dead	104,000	368,000	+++ Killed
2.25	113,000	240,000	Dead	95,000	352,000	+++ Killed
3.75	110,000	16,000	+ Killed	98,000	19,000	— Killed
5.0	117,000	—	— Dead	118,000	—	— Killed
7.0				121,000	—	— Killed

Arsphenamine

0.225	99,000	180,000	Dead			
0.375	112,000	22,000	Dead		21,000	+++ Killed
0.5	100,000	12,000	++ Killed	103,000	9,000	++ Killed
0.75	125,000	10,000	++ Killed	89,000	—	— Killed
1.0	158,000	—	— Killed	94,000		
1.5	129,000	—	— Killed	90,000	—	— Killed

Neosphenamine

0.375	94,000	12,000	Dead			
0.5	115,000	—	— Killed			
0.75	114,000	—	— Dead	165,000	90,000	+++ Killed
1.0	120,000	—	— Killed	93,000	—	— Killed
1.5	108,000	—	Dead	150,000	—	— Killed
2.25	199,000	—	— Killed	154,000	—	— Killed
3.75				148,000	—	— Killed

Sodium arsenate

7.5	158,000	31,000	Dead	160,000	568,000	+++ + Dead
10.0	136,000	—	— Killed	104,000	792,000	+++ + Dead
15.0	120,000	— Dead		174,000	360,000	+++ + + Dead
22.5	110,000	—	— Killed	116,000	3,500	+++ + + Killed
37.5				148,000	— Dead	

TABLE 2—*Concluded*

DOSE	URETERS LIGATED			CONTROLS		
	Initial count	24 hours	48 hours	Initial count	24 hours	48 hours
p-Oxy-m-aminophenylarsonic acid (sodium salt)						
cc.						
0.5	116,000	56,000	++ Killed			
0.75	173,000	—	— Killed			
1.0	100,000	—	— Dead			
2.25	120,000	—	— Dead			
3.75	126,000	—	— Dead			
5.0	100,000	—	— Dead	160,000	1,200,000	Dead
7.5	120,000	—	— Dead	110,000	624,000	Dead
10.0	120,000	—	— Dead			— Killed
15.0				112,000	6,000	— Killed
22.5				106,000	2,500	— Killed
37.5				104,000		— Killed
Arsacetin						
0.5	105,000	+++ Dead	— Dead			
1.0	120,000	—	— Dead			
1.5	172,000	—	+ Dead			
2.25	153,000	—	— Dead			
3.75	121,000	—	— Dead			
5.0	120,000	—	— Dead	125,000	872,000	Dead
7.5	141,000	—	— Killed	111,000	612,000	Dead
10.0	133,000	—	— Killed			Trace, Killed
15.0	136,000	—	— Dead	70,000	11,000	— Killed
22.5				74,000	25,000	— Killed
37.5				90,000	—	— Killed

Atoxyl

0.75	136,000	++++ Dead	- Dead		
1.0	127,000	213,000			
1.5	126,000	- Dead			
2.25	125,000	136,000	+ Dead		
3.75	110,000	65,000	+ Dead		
5.0	97,000	-	- Dead	109,000	880,000
7.5				100,000	696,000
15.0				120,000	125,000
22.5				100,000	-
37.5				99,000	-
					+ Dead
					+ Dead
					+ + + + Killed
					- Killed
					- Killed

Phenylglycin-p-arsonic acid (sodium salt)

5.0	118,000	260,000	- Dead		
7.5	91,000	256,000	+ Dead		
10.0	89,000	198,000	+ Dead		
15.0	97,000	568,000	+ Dead		
22.5	94,000	-	- Killed		
37.5	78,000	-	- Killed	89,000	+++ + + + Dead
50.0				96,000	1,224,000
75.0				86,000	656,000
150.0				80,000	552,000
225.0				108,000	-
375.0					+ Dead
					+ Dead
					- Dead
					- Killed

and it is interesting to note that the margin of safety of this compound is so great in animals with ligated ureters. This property undoubtedly contributes to the therapeutic usefulness of arsphenamine, when attention is called to the probable formation of this well tolerated metabolism product of arsphenamine in the animal body. We may conclude from these experiments that the effect of ligation of both ureters upon toxicity and trypanocidal action is in perfect agreement with the conclusions drawn from the previous study of the rate of urinary excretion of the arsenic of these compounds.

Effect of ligation of bile duct. Only three drugs were studied in this connection, as the principal purpose of this investigation was to establish the relation of rate of excretion and pharmacological action. In the preceding paper it had been shown that the greatest amount of arsenic appears in the feces after intravenous injections of the arsenobenzene compounds, whereas only a small amount of arsenic can be detected in the feces after the administration of the other arsenicals. For these reasons arsphenamine, neoarsphenamine and atoxyl were chosen for these experiments, the first two as drugs which presumably might be excreted with the bile to a considerable extent, the last being principally eliminated by the kidney.

Tables 3 and 4 show conclusively that the toxicity and trypanocidal action of atoxyl is not influenced to any extent whatever by ligation of the bile duct. This negative result was to be expected as the kidney excretes the arsenic of atoxyl with great speed. If any arsenic of this compound is normally excreted with the bile it must be a very small part of the total elimination and with the bile duct ligated would probably be taken care of by the kidney. Complete obstruction of the bile duct results in an appreciable increase of the trypanocidal activity of arsphenamine (6 times) and neoarsphenamine (2, 3 times). It is surprising however to find that the toxicity is not appreciably affected in the case of arsphenamine and only slightly with neoarsphenamine. The compensatory function of the kidney probably explains these facts. At any rate ligation of the bile duct does not seem to exert such a powerful influence upon the

TABLE 3
Effect of ligation of bile duct on toxicity

DOSE	BILE DUCT LIGATED			CONTROLS		
	Symptoms	Time of death	Autopsy	Symptoms	Time of death	Autopsy
Arsphenamine						
cc.		days			days	
15.0	Absent	S 4	Negative	Absent	S 4	Negative
22.5	Absent	S 4	Liver fatty	Absent	S 4	Negative
37.5	Absent	S 4			D 2	Kidneys pale; pleural cavity filled with bloody transudate
50.0		D 1	Slight amount of pleural fluid		D 2	
Neocarsphenamine						
22.5	Absent	S 3	Negative	Absent	S 2	Negative
37.5	Hyperexcitable	S 3	Negative	Absent	S 3	Negative
37.5		D 1	Intestines hemorrhagic			
50.0		D 1	Large intestines hemorrhagic	Absent	S 3	Negative
75.0	Sick appearance	D 1	Kidneys congested. Gastric and intestinal hemorrhage		D 2	Kidneys congested; liver fatty appearance
100.0	Sick appearance	D 1	Kidney degeneration. Stomach hemorrhagic		D 1	Stomach hemorrhagic
Atoxyl						
50.0	Droopy	S 4	Negative	Slight tremors	S 5	Negative
75.0	Absent	S 4	Negative	Ataxia, tremors	S 3	Negative
100.0	Ataxia, tremors	S 4	Kidneys yellowish-white	Ataxia, tremors	S 2	Negative
150.0	Ataxia, tremors	D 2	Negative	Ataxia, tremors	D 1	Small intestines slightly hemorrhagic

TABLE 4
Effect of ligation of bile duct on trypanocidal action

DOSE	BILE DUCT LIGATED			CONTROLS		
	Initial count	24 hours	48 hours	Initial count	24 hours	48 hours
Arsphenamine						
α .						
0.1	178,000	904,000	+++ Dead			
0.15	130,000	602,000	+++ Dead			
0.225	246,000	130,000	+++ Dead			
0.375	102,000	—	Trace, Killed			
0.5	164,000	—	— Killed	284,000	228,000	+++ Dead
0.75	170,000	—	— Killed	162,000	14,000	+++ Killed
1.0				170,000	110,000	+++ Killed
1.5				168,000	28,000	+++ Killed
2.25				220,000	Trace	— Killed
3.5				300,000	2,000	— Killed
Neosarsphenamine						
0.5	256,000	220,000	+++ Killed			
0.75	212,000	22,000	— Killed	216,000	26,000	+++ Killed
1.00	120,000	30,000	— Killed	156,000	90,000	+++ Killed
1.5	154,000	Trace	— Killed	120,000	38,000	+++ Killed
2.25	138,000	—	— Killed	100,000	20,000	+++ Killed
3.5	160,000	—	— Killed	160,000	Trace	— Killed
5.0	144,000	—	— Killed	106,000	Trace	Trace, Killed

Atoxyl						
5.0	150,000	800,000	+++++	106,000	28,000	++++ Killed
7.5	170,000	556,000	+++++	100,000	—	— Killed
10.0	128,000	56,000	+++++	128,000	—	— Killed
15.0	192,000	167,000	+++++	144,000	—	— Killed
22.5	250,000	36,000	+++++	—	—	— Killed
35.0	108,000	—	— Killed	—	—	— Killed
50.0	144,000	—	— Killed	—	—	— Killed

toxicity as ligation of the ureters. Whether or not some of the arsenic of these drugs is excreted directly into the intestinal canal without passing through the liver cannot be decided from the available evidence.

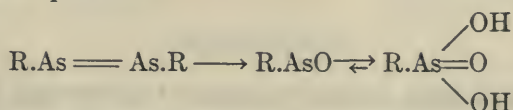
The net result of this investigation is to be found in the fact that direct proof is furnished for the theory advanced in the preceding paper. According to this theory the toxicity and parasiticial action of arsenicals is governed by the rate of

TABLE 5
Effect of ligation of both ureters on toxicity and trypanocidal action

DRUG	URETERS LIGATED			CONTROLS			RATE OF URINARY EXCRETION 24 HOURS
	Minimum lethal dose	Minimum effective dose	M.L.D. M.E.D.	Minimum lethal dose	Minimum effective dose	M.L.D. M.E.D.	
p - Oxy - m - aminophenylarsonoxide.....	7.5	0.375	20.0	7.5	0.375	20.0	13.3
Sodium arsenite.....	5.0	5.0	1.0	5.0	5.0	1.0	13.0
Arsphenamine.....	37.5	1.0	37.5	37.5	1.0	37.5	11.0
Neoarsphenamine.....	75.0	0.5	150.0	150.0	1.0	150.0	52.0
Sodium arsenate.....	10.0	10.0	1.0	37.5	37.5	1.0	51.0
p - Oxy - m - aminophenylarsonic acid.....	75.0	0.75	100.0	375.0	37.5	10.0	88.0
p - Acetylaminophenylarsonic acid (arsacetin).....	105.0	1.0	105.0	500.0	37.5	13.0	90.0
p - Aminophenylarsonic acid (atoxyl).....	5.0	5.0	1.0	150.0	22.5	6.7	93.0
Phenylglycin - p - arsonic acid.....	50.0	22.5	2.0	375.0	375.0	1.0	95.0

excretion of the arsenic from the body. It was shown that arsenicals which are rapidly excreted are also very little toxic and possess a low parasiticial value and vice versa. In other words a sufficiently prolonged retention of the drug by the tissues of the host is an essential requirement for the chemotherapeutic action of arsenicals. This fact disproves the truth of Ehrlich's assumption that drugs in order to be of therapeutic value must show very little or no affinity for the tissues of the host (organotropic properties). Examination of our experimental evidence clearly shows that the arsenic of arsphenamine and neoarsphenamine

mine is retained much longer by the tissues than that of the pentavalent oxidation product of arsphenamine, atoxyl or arsacetin. The rate of excretion in the case of arsphenamine and its substitutes is however considerably greater than that of the directly toxic trivalent arsenicals. It would therefore appear that the secret of the practical value of arsphenamine and its substitutes is to be found in the fact that these drugs per se are nontoxic and are deposited in the tissues immediately after injection. The deposited drug is then gradually converted by means of oxidation into the pharmacologically active trivalent "arsenoxide." This latter compound on account of its relatively slow and prolonged formation exerts its toxic effect upon the parasites and the tissues during a long time, this leading to the death of the parasites. Oxidation then proceeds a step further with the formation of pentavalent arsenic which can be readily eliminated from the body, principally by the kidney. This last reaction, namely the oxidation of the trivalent oxide to the pentavalent arsenic must be a reversible reaction (otherwise pentavalent compounds would prove ineffective), but the pentavalent arsenic is easily excreted, the arsenic once oxidized to this form disappears fairly rapidly from the body. This prevents a prolonged toxic action upon the tissues after the parasites have been killed, i.e., after the therapeutic effect has been obtained. These chemical changes are represented by the following formula in which the length of the arrows indicates roughly the rate at which these reactions proceed:



The question why the directly toxic and therapeutically active trivalent oxidation product of arsphenamine ("arsenoxide") is more injurious to the parasites than to the tissues remains to be elucidated. The most probable explanation is that the tissues are capable of oxidizing this compound to pentavalent arsenic, a change accompanied by detoxification, whereas the parasites do not possess this property or only to a slight degree, and are therefore,

killed by the same concentration of the active modification ("arsenoxide") which the tissues are able to resist. These considerations hold true only for therapeutic doses of arsphenamine, as detoxification by the tissues is obviously limited by the rules of mass action. This conception is supported by two observations made in this laboratory:

First, the fact that as the dose of arsphenamine is increased the time of the appearance of the toxic symptoms and the beginning of the therapeutic action (disappearance of trypanosomes from blood stream) is gradually shortened; this indicates a greater formation of "arsenoxide" in the unit of time with insufficient speed of oxidation to the pentavalent nontoxic form.

Second, the work of Hooper, Kolls and Wright (1921) has shown that "arsenoxide" produces the same general type of histopathological change in the organs as arsphenamine, the former substance however being seven and one-half times more toxic to the rat kidney than the latter. This marked difference between the effect produced by the same amount of arsenic given in the form of these two drugs is due to the fact that when "arsenoxide" is used the concentration in the body of this *directly toxic* agent is much greater than after arsphenamine which must first be oxidized to "arsenoxide" before it can exert its toxic action. Detoxification, due to further oxidation to the pentavalent form, would obviously be less effective in the case of "arsenoxide" on account of the shorter time available, hence the more toxic effect upon the tissues.

CONCLUSIONS

1. Ligation of both ureters increases toxicity and parasiticial action of those arsenicals which normally show a rapid rate of urinary excretion. The toxicity and parasiticial action of arsenicals with a low rate of urinary excretion in normal animals is not appreciably affected by ligation of the ureters.

2. Complete obstruction of the bile duct increases the parasiticial action of arsphenamine and neoarsphenamine, but not of atoxyl, and produces only slight changes in the toxicity of neoarsphenamine.

3. These observations furnish direct proof for the correctness of the theory advanced in the preceding paper and shows that the retention of arsenicals by the tissues of the host (organotropism in the broad sense) is a necessary requisite for drugs of practical chemotherapeutic value. This retention by the tissues must however be followed by such a chemical change (effected by the tissues), as ultimately to lead to the formation of arsenic in a form which can be easily excreted. The bearing of these conceptions upon the future development of chemotherapy and the search for drugs of practical value has been intimated in the preceding paper.

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INFLUENCE OF ARSPHENAMINE AND NEOARSPHEN- AMINE ON THE EPINEPHRINE CONTENT OF THE ADRENAL GLANDS

BALDWIN LUCKE, JOHN A. KOLMER, AND GRAYSON P. MCCOUCH

*From the Laboratories of Pathology and Physiology, University of Pennsylvania,
and The Dermatological Research Institute, Philadelphia*

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While studying the histological changes produced experimentally in rabbits by arspHENamine and by neoarsphenamine, definite lesions were observed in the adrenal glands (1, 2). The possible relation between such lesions and certain symptoms occurring occasionally after arspHENamine administration in man ("nitritoid crisis") led us to continue our investigation by means of combined physiological and morphological methods.

Previous research on the influence of arspHENamine and neoarsphenamine on epinephrine contents of the adrenal glands appears limited to the studies of Brown and Pearce (3) and of Hirano (4). The former writers studied the influence of a number of arsenicals on the lipoid and chromaffine substance of guinea-pigs, and of other laboratory animals, by histologic methods. They concluded that toxic doses of all the arsenicals used produce definite disturbances of the lipoid substances, reduction in the chromaffine contents and various cellular changes, and they suggested that therapeutic doses of some arsenicals may produce adrenal stimulation. Hirano (4) studied the adrenalin store of rabbits' adrenals after intravenous injection of salvarsan and neosalvarsan, employing the colorimetric method of Comesatti; he also investigated a number of glands histologically. He concluded that large doses of these arsenicals produced a remarkable decrease of chromaffine substance, and of residual epinephrine and that in therapeutic doses salvarsan caused partial exhaustion, while neosalvarsan did not influence the epinephrine contents.

METHOD OF EPINEPHRINE ASSAY

Rabbits of nearly uniform weight (1800 to 2200 grams) were given single or multiple injections of arsphenamine or neoarsphenamine, and after a certain time were killed by a blow on the head. The right adrenal glands were extracted according to the method of Folin, Dennis, and Cannon (5), and assayed by the biological method of Elliott (6). The left adrenal glands after fixation in Orth's fluid, were cut frozen, and from each gland one section was mounted unstained, one was stained with hematoxylin and eosin, and one with scarlet R.

The extracts for assay were brought to a constant of 15 cc. and were prepared just before the injection. It was found, however, that they did not lose any measurable percentage of epinephrine on standing at room temperature for one or two hours. As a standard, a 1:40,000 solution of adrenalin was used; this was prepared freshly before each experiment from pure adrenalin (kindly furnished by Parke, Davis and Company). We have satisfied ourselves by assaying solutions of adrenalin of known strength that the method of Elliott is very exact, but we agree with Sydenstricker, Delatour and Whipple (7) that assays of adrenalin are more apt to give clear cut results than assays of extracts, and that extracts may possibly contain substances that influence the reaction. During an experiment the same extract was usually assayed three or four times and the values obtained generally agreed fairly closely. It must be emphasized that there are a number of factors which are difficult to standardize and on which seem to depend the irregularities occasionally encountered. Thus we found that size and condition of the cats, the degree of shock sustained during their preparation, the degree of aeration, and the temperature of the warm pad upon which they were kept played a considerable rôle. We attempted to have conditions as nearly uniform as possible. Generally the right glands of two or three injected rabbits and of one control rabbit were assayed on one cat. Types of curves are illustrated in figures 1 and 2. The arsphenamine and neoarsphenamine used did not produce any symptoms in man, nor did the rabbits exhibit any noticeable reaction.



FIG. 1. EPINEPHRINE ASSAY OF EXTRACT OF RIGHT ADRENAL OF RABBIT XIII AND PRELIMINARY ASSAY OF EXTRACT OF RIGHT ADRENAL OF RABBIT XIX

Type of curve usually obtained. 1 cc. extract of right adrenal of rabbit XIII, was injected first, then 0.2 cc. of a standard solution of adrenalin, 1:40000. Since the curve of blood pressure fell below the extract curve, 0.3 of standard solution was injected next and this corresponded to the extract curve. The quantity of epinephrine in the extract is therefore 0.0075 mgm. times 15, or 0.113 mg. Since we generally superimposed 4 curves, a preliminary assay of extract XIX was made; the curve obtained rose above the two previous curves, and in another set of curves, the value of this extract was found to be 0.143 mgm. epinephrine. The abscissa indicates time in seconds, and serves also as baseline of zero pressure.

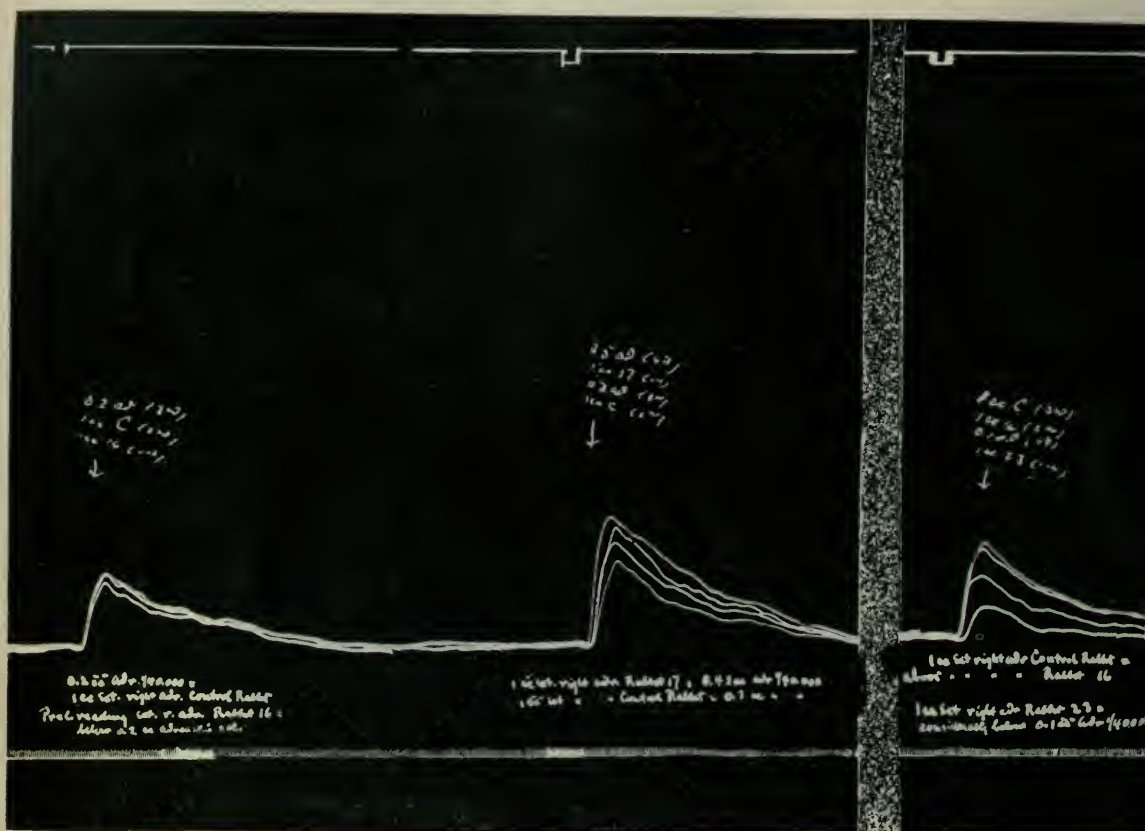


FIG. 2. EPINEPHRINE ASSAYS OF EXTRACTS OF RIGHT ADRENAL GLANDS OF RABBITS 16, 17, 23 AND ONE CONTROL RABBIT (C)

Top line = time of injection; abscissa = zero pressure and time in seconds. Read curves from topmost downwards; figures in parentheses indicate order of injection of extracts and of standard. First set of curves: 1 cc. of extract of right adrenal of rabbit 16 was injected first, and 1 cc. of extract of a control rabbit next; the second curve rose above the first and corresponded to 0.2 cc. of standard solution. Second set of curves: 1 cc. of extract of right adrenal of rabbit 17 was injected first, and 1 cc. of extract of the control rabbit next; the first curve topped the second, then 0.3 cc. and next 0.5 cc. of standard solution were injected; 1 cc. extract of rabbit 17 was calculated to equal 0.42 cc. of standard, or 0.157 mgm. epinephrine. Third set of curves: by interpolation the value of 1 cc. extract of right adrenal rabbit 23 which gave a very low curve was calculated to equal 0.05 cc. standard, or 0.019 mgm. epinephrine. The extract of the control rabbit topped the extract of rabbit 16 as in the first set of curves, the latter extract was calculated to equal 0.18 cc. standard or 0.068 mgm. epinephrine. Note that while the pressure is greater in the third set of curves the relation between extracts assayed parallels the first set of curves. These curves are exceptional, because the rise in pressure is small, but repetition of the assays gave almost identical results.

RESULT OF ASSAYS OF ADRENAL GLANDS OF NORMAL AND OF INJECTED ANIMALS

It has been shown for several species of animals (Elliott (8), Stewart and Rogoff (9), Sydenstricker, Delatour and Whipple (7), that the right and left adrenal glands contain approximately

TABLE 1
Epinephrine contents of adrenal glands of normal rabbits

DATE OF EXPERIMENT	WEIGHT OF ADRENALS	AMOUNT OF EPINEPHRINE IN GLAND	EPINEPHRINE PER 1 GRAM ADRENAL
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
June 27.....	R* 126	0.075	0.59
	L* 150	0.083	0.55
June 29.....	R 70	0.100	1.43
	L 80	0.140	1.75
June 30.....	R 224	0.112	0.50
	L 195	0.105	0.53
June 30.....	R 310	0.112	0.36
	L 340	0.112	0.33
July 6.....	156	0.150	0.96
July 7.....	272	0.075	0.28
July 11.....	114	0.094	0.82
July 12.....	290	0.218	0.75
July 19.....	142	0.038	0.27
July 22.....	155	0.105	0.68
August 27.....	280	0.094	0.33
September 9.....	125	0.075	0.60
Average.....	189	0.106	0.67

* R stands for right adrenal gland; L for left adrenal gland.

the same store of epinephrine. This is also true of the rabbit (table 1) and we are justified in comparing biological with histological observations. The assays of the normal glands are summarized in table 1. It can be seen that considerable variation exists in weight of glands as well as in the epinephrine store per gland, but on analysing the figures it is found that they fall fairly well within 50 per cent deviation of the arithmetic average,

namely, 0.106 mgm. In the third column the calculated quantity of epinephrine per 1 gram of gland is given. But since differences in weight of adrenal glands usually depend more upon cortical than upon medullary alterations (Elliott (8), Donaldson (10)), we feel that the custom of calculating the quantity of epinephrine per gram of adrenal gland is misleading. Therefore it seemed to us safer to draw conclusions from the actual

TABLE 2

Influence of a single injection of arsphenamine and neoarsphenamine on the epinephrine contents of adrenal glands

RABBIT NUMBER	DATE OF INJECTION	LENGTH OF SURVIVAL AFTER INJECTION	TYPE OF ARSPHENAMINE	DOSE PER KILO	WEIGHT OF RIGHT ADRENAL	AMOUNT OF EPI- NEPHRINE IN RIGHT ADRENAL	EPINEPHRINE PER 1 GRAM OF ADRE- NAL
				grams	mgm.	mgm.	mgm.
1	July 6	30 minutes	Arsphenamine	0.05	95	0.139	1.46
2	July 6	30 minutes	Neoarsphenamine	0.1	115	0.150	1.30
5	July 11	15 minutes	Arsphenamine	0.05	107	0.094	0.88
6	July 11	15 minutes	Neoarsphenamine	0.1	378	0.199	0.53
24	Aug. 22	15 minutes	Arsphenamine	0.02	270	0.083	0.31
25	Aug. 22	60 minutes	Arsphenamine	0.02	62	0.047	0.76
26	Aug. 22	15 minutes	Arsphenamine	0.03	190	0.094	0.49
27	Aug. 22	60 minutes	Arsphenamine	0.03	155	0.105	0.68
3	July 7	18 hours	Arsphenamine	0.05	82	0.188	2.29
4	July 7	18 hours	Neoarsphenamine	0.1	155	0.112	0.73
7	July 11	18 hours	Arsphenamine	0.05	86	0.075	0.88
8	July 11	18 hours	Neoarsphenamine	0.1	63	0.075	1.19
9	July 18	18 hours	Arsphenamine	0.05	130	0.086	0.66
10	July 18	18 hours	Neoarsphenamine	0.1	128	0.086	0.67
Average.....					144	0.109	0.916

amounts of epinephrine per gland. In table 2 are shown the epinephrine contents of adrenal glands of animals injected with a single dose of arsphenamine or neoarsphenamine; the doses used exceed from two to five times those corresponding to therapeutic doses. There is considerable range of values obtained but, in general, the amounts are similar to those of normal animals, and the total average, 0.109 mgm. epinephrine per gland, is almost identical with the average amount obtained for the

control rabbits. There seems to be no striking difference between the action of arsphenamine and of neoarsphenamine, and the factor of time of survival played apparently no definite rôle.

In table 3 the effect of multiple therapeutic doses of arsphenamine and neoarsphenamine are summarized. The average amount of epinephrine per gland is slightly less than that of the normal series or of the animals injected with a single dose but the number

TABLE 3

*Influence of multiple injections of arsphenamine and neoarsphenamine on epinephrine contents of adrenal glands**

RABBIT NUMBER	DATE OF INJECTION		LENGTH OF SURVIVAL AFTER INJECTION	TYPE OF ARSPHENAMINE	NUMBER OF DOSES	DOSE PER KILO		WEIGHT OF RIGHT ADRENAL	AMOUNT OF EPINEPHRINE IN RIGHT ADRENAL		EPINEPHRINE PER 1 GRAM OF ADRENAL
			days			grams	mgm.		mgm.		mgm.
12	July	29	1	Arsphenamine	22	0.01	110	0.064	0.58		
13	July	29	1	Arsphenamine	2	0.01	135	0.113	0.83		
15	August	27	8	Arsphenamine	6	0.01	360	0.094	0.26		
16	September	9	1	Arsphenamine	12	0.01	140	0.068	0.48		
17	September	9	1	Arsphenamine	12	0.01	373	0.157	0.42		
18	July	29	1	Neoarsphenamine	2	0.02	210	0.064	0.30		
19	July	29	1	Neoarsphenamine	2	0.02	240	0.143	0.60		
21	August	27	8	Neoarsphenamine	6	0.02	165	0.075	0.46		
23	September	9	1	Neoarsphenamine	12	0.02	174	0.019	0.11		
Average								212	0.088	0.44	

* The injections were made three or four days apart.

of injections or the type of arsenical used does not produce striking variations.

HISTOLOGICAL OBSERVATIONS

Sections prepared as indicated were studied for the quantity and the distribution of the lipoids which stained with scarlet R, and for the quantity and distribution of the medullary chromaffine substance. For descriptive purposes the customary division into three cortical zones is used.

Glands of normal animals. There was considerable variation

in proportion of medulla to cortex. In the zona glomerulosa of the latter, the lipid substances were almost absent or at the most very scanty. In the zona fascicularis the fat was sometimes fairly uniformly distributed, but frequently the outer one-half of this zone appeared to contain a greater quantity than the inner half. In the zona reticularis and in the innermost layer of the zona fascicularis the amount of lipid substances varied considerably. In the majority of the sections there were here scanty numbers of fine fat droplets, usually irregularly distributed. There was considerable variation in the size of the individual fat droplets; generally they were smallest when least plentiful, and largest when abundant. When scanty they appeared more at the cellular periphery, leaving a fairly clear area about the nucleus; in no instance were any fat substances present in the nucleus. There was no sharp dividing line between medulla and cortex, but everywhere there were small cortical projections and islands present in the medulla. These islands and projections usually contained lipid droplets. The medulla took on a fairly uniform yellow-brown stain, the intensity of which corresponded often, but not always, to the epinephrine contents in the right adrenal. There were, however, a number of glands where no parallelism seemed to exist. The sections were arranged in a series according to depth of color. This scale did not correspond to the true adrenalin values, and we were forced to conclude that while considerable variations in the epinephrine contents might be judged roughly by the depth of the brown chromaffine tint, slight and even moderate variations could not be determined by this method. This agrees with the reports of other observers (Biedl (11), Elliott (8)).

Gland of treated animals. There was so little difference between the glands of the treated groups and the normal glands that it is not worth while to detail results. In general those animals which had received a single injection usually showed a very slight increase of lipid substance, which appeared most marked in the fasciculate zones. The chromaffine reaction showed the same variations as in the normal animals; there was no parallelism with physiological assays. Animals which had received

multiple injections, particularly those with twelve injections, presented moderate reduction of the lipid substances. The cortex appeared paler on the whole, and here and there pale, irregular patches were present. The chromaffine substances, on the other hand, did not show sufficiently striking changes to allow conclusions to be drawn. There were no striking or definite differences between the glands of animals treated with arsphenamine or neoarsphenamine.

SUMMARY

The right adrenal glands of rabbits injected with single doses of arsphenamine and neoarsphenamine, in from two to five times the corresponding therapeutic amounts, were assayed for their quantitative epinephrine contents by the biological method of Elliott. A considerable range of values was obtained, but on the average, the amounts were almost identical with the average of the epinephrine values of control rabbits.

The right adrenal glands of rabbits injected with two to twelve "therapeutic" doses of arsphenamine and neoarsphenamine gave, on the average, epinephrine values slightly less than those of control rabbits. Of nine animals receiving multiple injections only one (no. 23) shows a reduction beyond the range of the controls. No striking difference was found between the influence of arsphenamine or neoarsphenamine, nor did the time of survival after injection appear to play any appreciable rôle. Histological studies of the corresponding left glands did not show any noticeable and constant alterations of the chromaffine reaction. The lipoids appeared very slightly increased after a single injection, and slightly decreased after multiple injections.

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A STUDY OF THE COLLOIDAL PROPERTIES OF ARSPHENAMINE AND ALLIED PRODUCTS¹

GEORGE W. RAIZISS AND JOSEPH L. GAVRON

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INTRODUCTION

According to the early literature, the cause of toxicity and "reactions" following the intravenous injections of arspenamine is due to the presence of an impurity, the oxidation product, 3-amino-4-hydroxyphenylarsenious oxide called "arsenoxide." Ehrlich and Bertheim declared "that arspenamine like other arseno compounds possesses the property of readily undergoing oxidation. Exposed to the air, it will soon contain aminohydroxyphenylarsenoxide; indeed the production of 'arsenoxide' takes place if the preparation is kept in an ordinary glass container. This fact is therefore of the greatest importance in the practical use of the above drug because the 'arsenoxide' is about 20 times more poisonous than arspenamine" (1).

In 1917, however, Schamberg, Kolmer and Raiziss (2) as a result of their investigations along the same lines, found that "arsenoxide" is not as toxic as originally claimed, but only from three to four times more so than arspenamine. At the same time, they stated, that after the quantitative chemical examinations of a number of different samples of arspenamine, it was evident that this impurity is present in but small amounts. This was subsequently confirmed again by Raiziss and Proskouriakoff by a different series of investigations (3). In addi-

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tion, Schamberg and his collaborators (2) claimed it is not so much 3-amino-4-hydroxyphenylarsenious oxide, but a small amount of a hitherto unknown very toxic substance X, together with factors related to the patient and to the technique of administration that are the causes of the disturbing phenomena associated with arsphenamine therapy.

Later, Reid Hunt (4) stated "that no toxic commercial preparations of arsphenamine have been encountered, the toxicity of which could be attributed to the presence of the 'oxide.'" The probability that there is present a substance or substances more toxic than arsphenamine itself, he regards as certain. But, in addition, the same investigator considers it highly probable that the toxicity is dependent to a considerable extent upon the "physical state of the solution, and that this undergoes rapid change when the solution is warmed." Similar observations were very recently made by Schamberg, Kolmer and Raiziss (5).

It occurred to the authors, that an investigation of the colloidal properties of arsphenamine might eventually aid in the solution of the problem. Hugo Bauer (6), as a result of his researches on some colloidal properties of silver and sodium salvarsans, as well as collargol and atoxyl, concludes that they belong to the class of semi-colloids and the sizes of the particles are such as represent the dividing line between the colloids and crystalloids. Silver arsphenamine, he claims, is a chemically uniform substance, the silver existing in complex combination with arsphenamine and not merely as a mechanically admixed colloid. He also performed diffusion experiments in a gelatine medium and draws therefrom an idea as to how the arsenicals behave in the organism.

M. Z. Klemensiewicz (7) in studying the viscosity of aqueous solutions of arsphenamine found that it increases rapidly until a constant value is reached. The greater the concentration, the more rapid is the rate of increase in viscosity as well as the viscosity constant. Therefore a very concentrated solution cannot be prepared as it gelatinizes. He also classes arsphenamine among the "lyophiles" or emulsoids which include gelatine, albumins, etc. He advances the theory that the increase in viscosity is due to polymerization of the arsphenamine.

That the dihydrochloride of 3,3'-diamino-4,4'-dihydroxyarsenobenzene is of a colloidal nature may be readily deduced from its behavior upon dissolving in water. Some preparations are more difficult to dissolve than others at room temperature; in hot water, however, they are very readily soluble. The phenomenon to be observed, when the drug is first introduced into water, is very characteristic. A viscous gelatinous mass is formed. Upon shaking, this breaks up into smaller aggregates, which swell and float upon the surface of the water. Moreover, these particles are enveloped in a film which interferes with the penetration and therefore solution by the solvent. It is sometimes necessary to resort to shaking with glass beads in order to obtain a solution. Warming or the addition of a little ethyl alcohol is of material assistance.

This property of gel formation by arsphenamine affords a ready comparison to gelatine and its well-known property of swelling due to the absorption of varying amounts of water. This swelling force seems to vary with different preparations, due perhaps to the variations in the methods of converting 3,3'-diamino-4,4'-dihydroxyarsenobenzene into the corresponding dihydrochloride. It is a known fact that the hydroxyl group exerts an inhibitory influence upon imbibition. This might serve as an explanation of the beneficial action of alcohol in dissolving arsphenamine.

Those who have had occasion to handle arsphenamine may have observed perhaps, its great power of adsorption. If a small quantity of charcoal is introduced into a methyl alcoholic solution of arsphenamine, a portion is completely adsorbed so that after filtering and precipitating the filtrate with ether, it dissolves completely in water together with the drug. Coloring matter and other substances are also readily adsorbed. This is one of the reasons why it is so difficult to obtain a perfectly pure sample of arsphenamine.

The true colloidal nature of arsphenamine is seen upon evaporating its aqueous solution in vacuo to a point of great concentration when a thick gelatinous mass is obtained. It can be drawn out in long shreds similar to glue, and is very diffi-

cult to redissolve by means of water. Arsphenamine behaves generally like a reversible colloid, in that when once precipitated from solution, it can be redissolved forming a colloidal solution in contradistinction to the proteins and various colloidal metals which belong to the class of irreversible colloids. The less soluble variety of the drug cannot be however converted into the more soluble. In this respect it is irreversible.

We were particularly interested in determining, whether or not arsphenamine still retains its colloidal properties when converted into the disodium salt since the latter and not the dihydrochloride, is used in therapy; also the very important drug neoarsphenamine; and if they are colloids, to what extent? We also included the study of silver and gold sodium arsphenamines.

EXPERIMENTAL

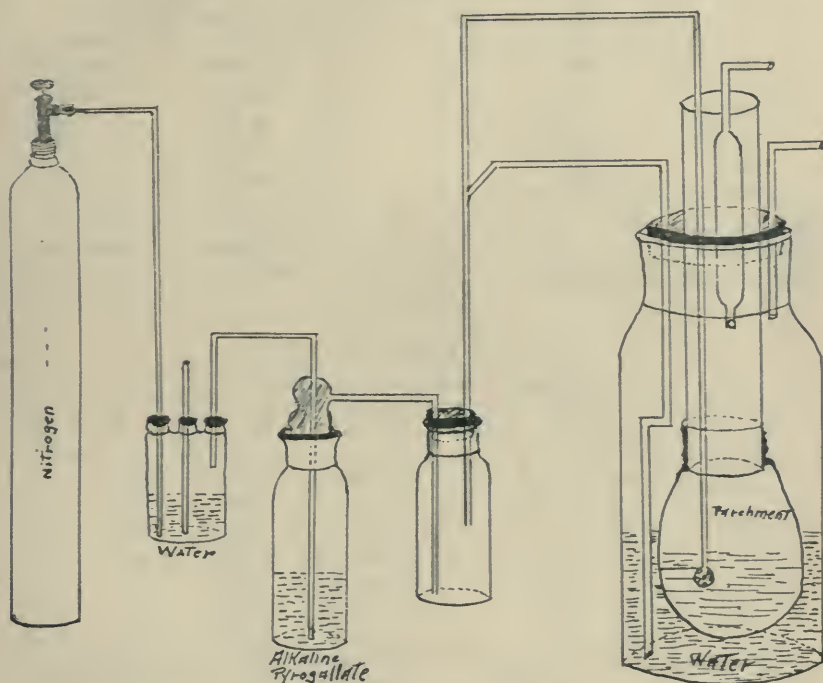
The same procedure as that employed by Graham, the founder of colloidal chemistry, in 1862, was followed, namely, diffusion through animal or vegetable membranes. Although this method is usually employed for mere qualitative experiments, it can be rendered a quantitative procedure if uniform conditions are observed, thereby furnishing some interesting data.

Our apparatus consists of a wide mouth bottle, of about 500 cc. capacity, fitted with a cork stopper with three perforations. Through one is inserted a bottomless test tube, 8 inches long and 1 inch in diameter, to which is attached a parchment bag.² In the top of the tube is a rubber stopper provided with two perforations, one containing a piece of narrow glass tubing extending below the surface of the liquid within the parchment bag for the entrance of nitrogen. The other serves as an outlet for the gas. The other two holes in the cork stopper of the bottle serve a similar purpose for the entrance into and exit of the nitrogen from the liquid surrounding the parchment. The gas was freed of oxygen before entering either of the two solutions, by being passed through an alkaline pyrogallate solution.

² The parchment used was that prepared by H. Reeve Angel and Company of New York City.

We first tested the permeability of the membrane to pure sodium chloride. It was dialyzed for thirty-six hours, the outer water being changed every six hours and analyzed for chlorine. In the first six hours 68.49 per cent diffused through, 88.31 per cent after twelve hours and 99.05 per cent after thirty hours.

In the dialysis of arspenamine, slightly more than 1 per cent of the arsenical diffused during the first six hours, while after



ARRANGEMENT FOR DIALYZING IN AN ATMOSPHERE OF NITROGEN

thirty-six hours 9.16 per cent was found in the combined dialysates. Determinations of the iodine values or oxygen absorbing capacities of the individual dialysates as well as the arsenic analyses by the method of Lehman and the ratios of these values served to substantiate the fact that the arsenical present was arspenamine. The values obtained for the ratios indicated in table 2 are in close agreement with that obtained for the original sample. The slight diffusion of arspenamine proves that it possesses distinct colloidal properties.

Upon repeating this same experiment, using methyl alcohol instead of water and precipitating the dialysate with ether, we obtained a yellow precipitate. When analyzed for arsenic,

TABLE 1
Dialysis of sodium chloride in water

TIME	Cl	Cl
<i>hours</i>	<i>mgm.</i>	<i>per cent</i>
6	415.00	68.49
12	120.30	19.82
18	42.33	6.97
24	16.39	2.70
30	6.51	1.07
36	Negative	Negative
Total.....	600.53	99.05
Residue.....	Negative	Negative
Calculated for 1 gram NaCl.....	606.80	

TABLE 2
Dialysis of arsphenamine in water

TIME	As	As	O	$\frac{\text{As}}{\text{O}}$
<i>hours</i>	<i>mgm.</i>	<i>per cent</i>	<i>mgm.</i>	
5	12.93	1.30	5.33	2.42
12	23.40	2.36	8.75	2.66
18	20.05	2.02	6.48	3.09
24	15.06	1.52	5.31	2.83
30	9.69	0.98	3.81	2.54
36	9.69	0.98	3.81	2.54
Total.....	90.82	9.16	33.49	2.68
Residue*.....	824.56	83.26	342.72	2.40
Calculated for 3.3 grams.....	990.33		424.51	2.33

* The residue could not be quantitatively removed from the bag, which accounts for the discrepancy in the sum total of arsenic found in the combined dialysates and the residual liquid within the bag.

nitrogen and iodine or oxygen absorbing value it proved to be arsphenamine. This was particularly gratifying in that it demonstrated, that we were able to prevent oxidation during the dialysis. Moreover, 24 per cent of the arsphenamine diffused

through in but eighteen hours, which is *four times* the amount obtained with water under the same conditions.

From the results tabulated in table 4, it appears that the hydrochloric acid of the arspenamine was split off and diffused much more rapidly than the resulting arsenical compound. Particularly significant is the fact that 43.8 per cent of the chlo-

TABLE 3
Dialysis of arspenamine in absolute methyl alcohol

TIME	As	As
<i>hours</i>	<i>mgm.</i>	<i>per cent</i>
6	30.82	9.90
12	18.7	6.01
18	29.1	9.35
Total dialyzed.....	78.6	25.25

TABLE 4
Dialysis of arspenamine in water

TIME	Cl	Cl
<i>hours</i>	<i>mgm.</i>	<i>per cent</i>
6	61.8	43.82
8	28.1	19.92
10	17.9	12.69
12	12.6	8.93
18	12.6	8.93
24	5.8	4.11
30	4.9	3.47
Total after thirty hours.....	143.7	
Residue.....	0.00	0.00
Original analysis.....	141.0	

rine was found in the dialysate of the first six hours, and also that the residue after thirty hours was entirely free of the same.

In order to determine whether the loss of hydrochloric acid affected the diffusion of the arsenical, an experiment was performed in which the acid found in the dialysate at the end of every six hours, was replaced by an equal amount of standard hydrochloric acid.

It can be seen that the rate of diffusion is about the same as in the previous experiment in which the acid was not replaced (table 2), the percentages of arsenic amounting to 10.61 and 9.16 per cent, respectively.

TABLE 5
Dialysis of arsphenamine in water replacement of diffused acid

TIME	As	As	HCl
hours	mgm.	per cent	mgm
6	29.61	2.89	67.88
12	24.05	2.35	67.88
18	13.67	1.33	63.88
24	14.85	1.45	76.21
30	13.89	1.35	71.90
36	12.60	1.23	79.86
Total.....	108.67	10.61	
Calculated for 3.3 grams arsphenamine.	1023.00		

TABLE 6
Dialysis of disodium arsphenamine in water

TIME	As	As	O	O	$\frac{\text{As}}{\text{O}}$
hours	mgm.	per cent	mgm.	per cent	
6	34.26	3.35	14.82	3.46	2.31
12	84.27	8.23	29.84	6.97	2.82
18	82.42	8.05	28.07	6.55	2.93
24	62.03	6.06	21.03	4.91	2.94
30	45.35	4.43	17.00	3.97	2.66
36	55.55	5.43	17.00	3.97	3.26
Total.....	363.89	35.55	127.76	29.83	2.84
Residue.....	481.60	47.07	187.78	43.87	2.56
Calculated for 3.3 grams arsphenamine.	1023.00		428.01		2.39

When arsphenamine in aqueous solution is converted into its disodium salt with caustic soda and the resulting solution dialyzed, 35.55 per cent of the arsenical diffused as compared to 9.16 per cent in the case of the dihydrochloride.

The arsenic to oxygen ratios in the last column show that no oxidation occurred during the experiment. Particularly strik-

ing in this respect is the As:O ratio of the residue, 2.56, as compared to that of the original, 2.39. The alkali diffuses through somewhat faster than the arsenical.

The alkalinity of the first dialysate, shows that during the first six hours considerably more sodium ions passed through the membrane than that required by the quantity of disodium salt corresponding to the amount of arsenic which diffused during the same time. The theoretical As:Na ratio for the original sample is 3.28, while that found for the first six hours is 0.68; the ratios for the succeeding dialysates are invariably less than the theoretical. The total arsenic which diffused during thirty-

TABLE 7
Dialysis of disodium arspenamine in water

TIME	As	As	NaOH	NaOH	$\frac{\text{As}}{\text{Na}}$
hours	mgm.	per cent	mgm.	per cent	
6	31.48	3.07	80.60	13.56	0.68
12	73.16	7.15	47.16	7.94	2.68
18	82.44	8.05	51.12	8.60	2.80
24	57.34	5.61	35.38	5.94	2.81
30	52.78	5.16	31.45	5.29	2.92
36	49.08	4.79	27.52	4.63	3.10
Total.....	346.28	33.83	273.23	45.96	
Calculated for 3.3 grams arspenamine.	1023.00		594.00		6.52

$$\text{Ratio } \frac{2\text{As}}{2\text{Na}} = 3.26; \frac{2\text{As}}{\text{Na}} = 6.52$$

six hours shows close agreement with that of table 6, thereby indicating the degree of uniformity obtainable by the method employed.

That the greater diffusibility of the sodium does not affect that of the arsenical, an experiment was performed in which the alkali passing through every six hours was replaced by an equal amount of 0.1 normal sodium hydroxide. As seen in table 8, the rate of diffusion increased but slightly, 39.45 per cent of the arsenical diffusing instead of 35.53 per cent.

Five grams of neoarsphenamine containing 0.963 gram of arsenic was dissolved in water and dialyzed exactly like the

arsphenamine. After thirty-six hours, 26.1 per cent of the arsenic compound had diffused. Determinations of the oxygen requirement indicated this to be an arseno compound. At the same time 65.9 per cent of the original sulfur content was found in the dialysate.

TABLE 8

Dialysis of arsphenamine (disodium salt) in water (replacement of diffused alkali)

TIME	As	As	NaOH
hours	mgm.	per cent	mgm.
6	64.35	6.29	47.80
12	91.30	8.92	51.76
18	67.60	6.60	44.20
24	70.38	6.88	51.80
30	64.84	6.33	47.60
36	45.35	4.43	42.32
Total.....	403.82	39.45	
Calculated for 3.3 grams arsphenamine.	1023.00		

TABLE 9

Dialysis of neoarsphenamine in water

TIME	As	As	SULFUR	SULFUR
hours	mgm.	per cent	mgm.	per cent
5	36.70	3.81	76.50	17.21
12	62.21	6.46	63.70	14.31
18	56.97	5.91	63.40	14.26
24	36.93	3.84	43.75	9.84
30	31.89	3.20	30.88	6.94
36	27.74	2.88	14.70	3.30
Total.....	252.44	26.10	292.93	65.90
Calculated for 5 grams of neoarsphenamine.....	963.00		444.50	

A solution of disodium 3-amino-4-hydroxyphenylarsonate was dialyzed for thirty-six hours in exactly the same manner as the previous compounds. After six hours, 32.12; eighteen hours, 73.23 and thirty-six hours, 91.49 per cent of the arsenic had passed through the membrane. The average arsenic to nitrogen ratio was 5.02 as compared to the theoretical value 4.96.

5.809 grams of silver sodium arspenamine, as prepared by the authors and containing 1.076 grams of arsenic, was dissolved in water and dialyzed. Qualitative tests of the dialysates after every six hours for silver proved negative in all instances. The residual liquid within the parchment bag upon analysis was found

TABLE 10

Dialysis of disodium 3-amino-4-hydroxyphenylarsonate in water

TIME	As	As	N	N	$\frac{As}{N}$
hours	mgm.	per cent	mgm.	per cent	
6	307.44	32.38	60.24	31.47	5.10
12	259.37	27.31	50.02	26.13	5.18
18	132.32	13.93	26.13	13.70	5.06
24	92.01	9.69	18.07	9.44	5.09
30	53.78	5.66	12.33	6.44	4.36
36	32.74	3.44	7.78	4.06	4.02
Total.....	877.66	92.41	174.57	91.24	5.02
Calculated for 3.1 grams of the free acid.....	949.39		191.40		4.96

TABLE 11

Dialysis of silver sodium arspenamine in water

TIME	As	As	SILVER	SILVER
hours	mgm.	per cent	mgm.	per cent
6	90.70	8.42	Negative	Negative
12	55.66	5.17	Negative	Negative
18	51.91	4.82	Negative	Negative
24	38.86	3.60	Negative	Negative
30	29.17	2.71	Negative	Negative
36	23.43	2.17	Negative	Negative
Total.....	289.73	26.89	Negative	Negative
Residue.....	749.98	69.67	796.00	95.69
Calculated for 5.809 grams.....	1076.41		831.82	

to contain 95.7 per cent of its original silver content, the remaining 4.3 per cent adhering to the inner surface of the parchment and could not be quantitatively removed. The silver at the end of the experiment was present as a fine black precipitate, while the dialysates resembled in color those obtained

in the case of disodium arsphenamine. In thirty-six hours, 26.19 per cent of the arsenic had diffused.

By repeating this experiment with a sample of the same drug from another laboratory, after thirty-six hours 48.42 per cent of the arsenic had diffused, after eighty-four hours 86.77 per cent. As in the previous case, no silver was found in the dialysates at any time.

TABLE 12
*Dialysis of silver sodium arsphenamine in water**

TIME	As	As	Ag	Ag	O	$\frac{\text{As}}{\text{O}}$
hours	mgm.	per cent	mgm.	per cent	mgm.	
6	8.33	8.77	0.00	0.00		
12	12.93	13.58	0.00	0.00		
18	7.72	8.10	0.00	0.00		
24	5.56	5.84	0.00	0.00		
30	5.05	5.30	0.00	0.00		
36	5.55	5.83	0.00	0.00		
42	7.40	7.77	0.00	0.00		
48	6.48	6.49	0.00	0.00		
54	5.10	5.35	0.00	0.00	1.08	4.72
60	4.15	4.35	0.00	0.00	1.08	3.84
66	4.16	4.35	0.00	0.00	0.56	7.42
72	2.78	2.92	0.00	0.00	0.59	4.70
78	4.63	4.86	0.00	0.00	0.59	7.84
84	2.77	2.91	0.00	0.00	0.51	5.43
Total.....	82.61	86.77	0.00	0.00		
Calculated for 0.5 gram.....	95.20					

*This sample is the product of another laboratory.

In the dialysis of gold sodium arsphenamine, none of the gold passed through the membrane, similar to the case of the silver compound. After six hours, 8.62 per cent of the arsenic was present in the dialysate.

DISCUSSION

Bauer (6) as a result of his observations in diffusion experiments arranged the compounds studied according to the following: 1, collargol; 2, sulfoxylate (preparation 1495); 3, silver sodium arsphenamine; 4, neoarsphenamine; 5, arsphenamine; 6, alkalinized arsphenamine (disodium salt); 7, sodium arsphenamine.

mine (Höchst); 8, atoxyl, and 9, silver nitrate in which collargol diffuses least and silver nitrate most rapidly. According to our results, the following compounds arrange themselves thus: 1, arspenamine; 2, neoarsphenamine; 3, silver sodium arspenamine; 4, disodium arspenamine; 5, gold sodium arspenamine; 6, disodium 3-amino-4-hydroxyphenylarsonate. Comparing the results obtained for the first twelve hours, we found that arspenamine dialyzes about twenty-five times slower than sodium chloride. In methyl alcohol, however, the former diffuses four times more rapidly than in water, probably due to the ability of the alcohol to break up the drug into smaller particles.

Arsphenamine is apparently readily hydrolyzed in aqueous solution, since it was found that its chlorine ions diffused much more rapidly than the arsenical compound. Thus, after thirty hours there was no chlorine within the parchment bag while 81.14 per cent of the arsenical still remained. The authors observed a similar hydrolysis with the corresponding sulfate. This is insoluble in water, but by suspending in water and subsequently filtering, all of the sulfuric acid was found in the filtrate. In this diffusion experiment, the arsenical remaining in solution within the parchment was 3,3'-diamino-4,4'-dihydroxyarsenobenzene. This is surprising, since otherwise this compound is insoluble in water.

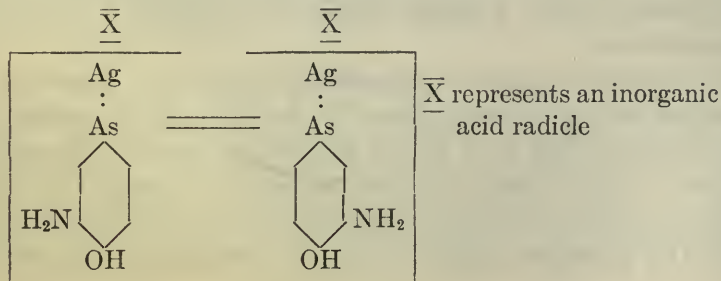
In the dialysis of an alkalized solution of arspenamine (disodium salt), the sodium ions diffused more rapidly than the arsenical. This phenomenon of the hydrolysis of alkaline salts of weak acids has been investigated and explained as membrane hydrolysis by Donnan and Harris (8) who state that "the acid is formed by the outward passage of the sodium ions along with the hydroxyl ions, leaving behind an equivalent number of hydrogen ions."

Although the more therapeutically important disodium salt of arspenamine is more diffusible than the dihydrochloride, it still possesses distinct colloidal properties, passing through the membrane eight times as slow as sodium chloride and almost four times as slow as disodium 3-amino-4-hydroxyphenylarsonate. These two arsenicals differ in that the arsenic is in the pentava-

lent form in the arsonate and in the trivalent condition in the arseno compound. Thus the *trivalence* of the arsenic seems to be an important factor with regard to both the therapeutic and colloidal properties of arsenicals.

Neoarsphenamine, which is so very soluble in water, possesses marked colloidal properties. In the first twelve hours but 10.27 per cent of the arsenical diffused as compared to 88.31 per cent of sodium chloride in the same time. The figures found for sulfur in the dialysates are very interesting. As has been shown by Raiziss and Falkov (9), neoarsphenamine contains varying amounts of uncombined sodium formaldehyde sulfoxylate. Assuming that as an average, 80 per cent of the total sulfur in the drug is combined to the amino groups and 20 per cent uncombined, it was found after dialyzing for thirty-six hours, that all of the free sulfur, 57.43 per cent of the combined sulfur, and only 26.1 per cent of the total arsenic passed through (table 8). This may be explained by the fact that the sodium formaldehyde sulfoxylate is but loosely bound to the amino group and therefore easily split off in aqueous solution. It is possible that similar hydrolysis occurs in the animal body, the amino groups being thus free to exert their original therapeutic energies. The introduction of substituents more firmly bound to the amino groups, as accomplished by Raiziss and Gavron (10), results in compounds possessing less therapeutic activity than neoarsphenamine.

The results obtained with silver and gold sodium arsphenamines are interesting because they throw a new light upon the chemical constitution of these more recent and apparently therapeutically important drugs. According to Ehrlich and Karrer (11) silver is attached to the arsenic thus:



while Binz, Bauer and Hallstein (12) maintained that the silver is attached to the amino groups thus:



H. Bauer (6) found that silver arsphenamine is a homogeneous substance easily passing through the ultrafilter, but not readily diffusible through a membrane. According to his results, it diffuses less readily than either neoarsphenamine or arsphenamine. Our results are not in accord with these findings. Although 26.89 per cent of the arsenic dialyzed, no silver was found in the dialysate. All of the metal remained within the parchment. This failure of the silver to pass through the membrane we noted with our own preparation as well as with a commercial sample. The latter was continued for eighty-four hours and involved the collection of 14 separate dialysates, each representing six hours' diffusion. Arsenic was determined and silver tested for in each. A total of 86.77 per cent of arsenic was found but not even a trace of silver. All of the latter was found within the parchment bag.

Similar results were obtained with the corresponding gold compound prepared by us. There are two possible explanations of this phenomenon. One is that the metal is combined either to the arsenic or nitrogen, forming a homogeneous chemical compound, during the dialysis of which the metal is again split off. Another view is, that we are dealing with mixtures of sodium arsphenamine and colloidal metals. We are inclined to accept the latter theory. The colors of the above two metallic arsenical compounds in aqueous solutions strongly resemble those of colloidal silver and gold respectively. The solution of the silver compound has a very deep brown, almost black, color resembling argyrol, while that of the gold compound is wine red. Upon standing twenty-four hours the latter deposited metallic gold. The method of preparation of these metallic

compounds possibly involves a reduction of the silver or gold salt to the metallic condition by either the arseno or the ortho aminophenol grouping and is held in colloidal suspension by the stabilizing effect of the disodium arsphenamine. The latter acts as a protective colloid just like dextrine, gelatine or casein in the formation of colloidal silver or gold solutions.

In the study of the toxicity and therapeutic effects of arsphenamine and its derivatives we are often confronted with phenomena which cannot be explained on the basis of our present chemical knowledge of these compounds. Indeed, Ehrlich had once declared that changes are very apt to occur in arsphenamine which are so delicate as to remain undetected by chemical means, the only tests being of biological nature. This statement has been confirmed by one of us in previous publications (2, 3 and 5). We are still unable to explain or overcome the peculiar phenomenon that apparently the same chemical processes yield arsphenamine, which sometimes is not tolerated in a dose of 100 milligrams per kilo of body weight and then again is borne in a dose as high as 170 milligrams. Although we are still searching for some tangible impurity which may be isolated and identified, it is our idea that colloidal investigations may eventually throw light upon this subject. As an illustration, it has already been established that alkaline solutions of arsphenamine upon standing or warming show a decreased toxicity. We maintained such solutions in an atmosphere of inert gas, free of oxygen, for long periods of time. Oxygen absorption determinations showed that no oxidation had occurred, yet upon testing their toxicities and therapeutic values it was found that marked changes had taken place. It is possible that this was caused by some change or changes in the size of the molecules or other colloidal properties of the solution.

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THE SO-CALLED HABITUATION TO "ARSENIC:" VARIATION IN THE TOXICITY OF ARSENIOUS OXIDE¹

ERICH W. SCHWARTZE

*Pharmacologist in Charge, Pharmacological Laboratory, Bureau of Chemistry,
United States Department of Agriculture*

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The observations made by the writer during the past three years in the course of approximately one thousand toxicity experiments with arsenious oxide, seem to explain, in part at least, the variations in acute toxicity of undissolved arsenious oxide as reported by various investigators. As far as the writer is aware, this is the first communication in which it is definitely recognized that more than one preparation of undissolved arsenious oxide has been used in a given study, although many isolated reports, from which the conclusions reached here may be inferred, exist in the literature. The great variation in the potency of different arsenious oxide preparations, when administered undissolved, seems to be dependent, at least in great part, upon the state of the subdivision of the oxide. The finer the powder the more toxic the preparation and vice versa. This was found to be true not merely of the toxicity but also of the emetic potency.

Moreover, the writer's observations, in spite of the fact that they pertain only to acute intoxications experimentally produced, are of such a nature as to raise the question whether or not real habituation to "arsenic"² ever actually occurs. These observa-

¹ The argument presented in this paper, submitted in brief on April 7, 1921, to Dr. C. L. Alsberg, Chief Chemist, Bureau of Chemistry is mentioned in his Annual Report for 1921. Preliminary communication was read at the meeting of the American Society for Pharmacology and Experimental Therapeutics held at New Haven, Connecticut, December 28, 29 and 30, 1921.

² The term "arsenic" is used in medical literature in a rather loose sense, meaning arsenious oxide, sometimes the sulphide.

tions, as will be shown, are in harmony with the recorded facts, but are out of harmony with the usual interpretation, namely, that these facts prove that habituation to "arsenic" can exist.

This argument will appear in two parts. The first gives a summary of the results of experiments made by the writer and the related data of other investigators, and the second presents an analysis of the evidence of habituation to "arsenic," which is based upon the facts given in the first part.

I. THE ACUTE TOXICITY OF ARSENIOUS OXIDE

Results of previous investigations

Mackenzie (19) recovered in the feces of a man who had attempted suicide two lumps of arsenious oxide weighing 7 grams (105 grains). He speaks of their "extreme insolubility."

Witthaus and Becker (34) state that careful search of "the medical and pharmaceutical periodical literature had failed to bring to light any record of the clearly established death of an adult from a dose of less than 2 grams (30 grains) of arsenic in the solid form." Many toxicologists admit, or permit the inference, that arsenious oxide in solution has a much greater toxicity than this (34) (12) (29).

Doyon and Morel (7) administered crystalline arsenious oxide in large doses to dogs, in one case 18 grams to a dog weighing 16 kilograms, without producing any ill effect. They concluded that the crystalline arsenious oxide was not absorbed.

Healey and Dimock (13) allude to the popular belief that hogs are not very susceptible to arsenical poisoning. They administered "enormous" doses (presumably of the undissolved oxide), as a result of which they concluded that "It would appear * * * that young hogs possess a marked tolerance" to arsenious oxide.

Edgar Everhart, a chemist of Atlanta, Georgia, states that he weighed out doses varying from $\frac{1}{2}$ to 16 grains (0.03 to 1 gram) of arsenious oxide, which were administered to dogs in a gum arabic suspension by a local physician. No ill effects were produced.³

³ Personal communication to the writer.

De Busscher (2) studied the toxicity of both dissolved and undissolved arsenious oxide. His results show that the preparation of arsenious oxide which he used, when administered undissolved, happened to be more toxic than that used by the other investigators here quoted and approached in degree the toxicity of dissolved arsenious oxide itself.

Willberg (33) was unable to confirm the low toxicity of undissolved arsenious oxide to dogs found by Doyon and Morel (7). Likewise he found the oral lethal dose on the chicken lower than that reported by Hausmann (10).

Green (9) studied the acute toxicity to sheep of a single preparation of undissolved arsenious oxide. The elimination was mainly by the feces, whereas administered in the dissolved state and in the form of sodium arsenite, the elimination was performed chiefly by the kidneys. As a result of a solubility test he decided that it was "the rate of solution and not the extent of solubility which is the governing factor in the absorption" of his preparation when it was administered undissolved. Green (9) appears to have been the first investigator to consider the fineness although he apparently used only one preparation.

The results of investigations already given, as well as many not reviewed here, show that the toxicity of undissolved arsenious oxide varies greatly. The fact that there is a disagreement concerning the toxicity of the oxide administered to the same species of animal is, however, sufficient to show the fallacy of assigning the entire ability of an animal to withstand the effects of a large amount of arsenious oxide to its species peculiarities. This fallacy is further shown by the results of the experiments of Willberg (33) and many others who have used the intravenous or subcutaneous method to study the resistance of several species against dissolved arsenious oxide or sodium arsenite. Without discounting the possibility of slight error in using data which might not be strictly comparable, the differences in resistance observed between species of higher animals are at most very small compared with those recorded for the oral administration of undissolved arsenious oxide.

Obviously, the ability of an animal to survive a large amount of undissolved arsenious oxide administered orally must be sought on other grounds than mere species resistance. Since it is improbable that all the investigators could be wrong as to size of the doses administered, attention was turned by the writer to the toxicity of different preparations of the arsenious oxide, and to the hypothesis of Green (9) that "the rate of solution * * * is the governing factor in absorption."

EXPERIMENTAL WORK

1. Toxicity of arsenious oxide

Rats. From observations on many rats the certain fatal dose of dissolved arsenious oxide administered orally was found to be about 75 mgm. per kilo. The certain fatal dose of undissolved arsenious oxide ground to an impalpable powder, the individual particles of which measured approximately from 0.0125 to 0.0025 mm. in diameter, is about 100 mgm. per kilo. This was found to be true of two separate preparations.⁴ One of these preparations was made from octahedral crystals of arsenious oxide (presumably sublimed). The other was prepared from the so-called opaque variety, which, however, proved to be crystalline in that it has the same index of refraction as the octahedral form.⁵ The certain lethal dose of a mixture of arsenious oxide crystals, of which most measured approximately from 0.25 to 0.125 mm. in diameter is about 350 mg. or slightly more per kilo. The lethal dose of crystal conglomerations, measuring approximately from 0.25 to 0.3 mm. in diameter, is about 500 mgm. per kilo. The two most potent preparations of undissolved arsenious oxide are therefore about five times more toxic to rats than the preparation which was least potent.

Rabbits. The certain fatal dose of the dissolved arsenious oxide administered orally, is more than 15 mgm. but not more

⁴ A patent for these preparations and their manufacture has been applied for. If granted, this will be dedicated to the free use of the Government and the public.

⁵ Examination made by Dr. E. T. Wherry, Crystallographer of the Bureau of Chemistry.

than 20 mgm. per kilo; for each of the finest preparations (0.0125 to 0.0025 mm.) referred to, about 25 mgm. per kilo; and of the 0.25 to 0.125 mm. crystals, about 200 mgm. (or slightly more) per kilo. Eight times as much of the less toxic preparation as of the two more toxic preparations of undissolved oxide, therefore, is required to produce death.

Chickens. The certain lethal dose of the dissolved arsenious oxide administered orally is certainly more than 66.7 mgm. per kilo; for each of the two finest preparations of arsenious oxide, about 75 mgm. per kilo; and for the 0.25 to 0.125 mm. preparation, about 200 mgm. per kilo. The more toxic of these solid preparations is only about two and one-half times as potent as the less toxic one.

2. *Emetic potency of arsenious oxide*

Emesis in cats was used as an index of potency, since the determination of a precise oral lethal dose in animals that vomit is usually not an easy procedure. In a forthcoming paper, Alsberg and Schwartz will show that the local emetic action of cadmium and zinc is best calculated on the basis of the concentration of these substances in the food. Although arsenious oxide is not a good emetic in that small doses produce very late and comparatively slight vomiting, the results obtained agree well with the basic features established for cadmium and zinc. It was, therefore, profitable to use this method for studying the potency of the large crystals which were not well suited for study by other methods.

The results of the vomiting experiments are given in table 1. From 90 to 95 per cent by weight of the very large crystals (series 6 and 7) was recovered in the vomitus and feces after soaking and washing the debris with running water. One cat consumed 2 grams per kilo without vomiting (series 7 (*experiment of the third day*)). This amount of arsenious oxide is six times the maximum amount which has been recorded as being consumed by arsenic eaters (25). They were the largest particles (crystals) used, and were at most only one-four hundred and twentieth as potent as dissolved arsenious oxide.

TABLE 1

Emetic potency of various preparations of arsenious oxide, according to the concentration in the diet, when administered in fair-sized meals of fresh meat to cats

SERIES NUMBER	ARSENIOUS OXIDE			MAXIMUM SUB-EMETIC CONCENTRATION		PRACTICALLY CERTAIN EMETIC CONCENTRATION	
	Prepara- tion *	Form or state	Approximate range in size of majority of particles	Parts per million in the food	Relative potency in terms of dissolved arsenious oxide†	Parts per million in the food	Relative potency in terms of dissolved arsenious oxide†
			mm.		per cent		per cent
0	A and B	Dissolved		35§	100.00	75§	100.00
1	B	Crystals and lumps	0.125 to 0.25‡	500§	7.00	1000§	7.50
2	B	Lumps	0.25 to 0.3	1000§	3.50	2000§	3.75
3	B	Lumps	0.3 to 0.6	Less than 2000§	More than 1.75	4000§	1.87
4	B	Lumps	0.4 to 0.6	2000	1.75		
5	D	Crystal chips	0.4 to 0.6			4000	Less than 1.87
6	D	Crystals	2 to 3			24,000	Less than 0.31
7	D	Crystals	2.5 to 5			31,600	Less than 0.24

* Letters in this column refer to the original source of material.

† Dissolved arsenious oxide as a standard, taken as 100 per cent.

‡ It was impossible to remove particles smaller than 0.125 mm. from this preparation. There were, however, relatively few of them.

§ These concentrations were arbitrarily selected for study. Their effects were compared with intermediate and greater concentrations.

|| Only a few sensitive cats were used, their behavior indicating that the certain emetic concentration for most cats had not been reached.

3. Potency of arsenious sulphide

The natural mineral orpiment obtained from Utah was used. Eight grams consisting of lumps ranging from 2 to 8 mm. in diameter were fed to a kitten without effect. About 7.5 grams of this material was mechanically recovered from the feces in the course of three days. Many particles had become fractured either in the eating or washing and sifting of the particles out of the feces. Many were very fine and too small to be separated from the débris which went through the sieve. This amount of orpiment appears to be about twenty-four times as great as the largest dose of "arsenic" which an arsenic eater has been reported as eating (25).

A sample of the same orpiment was mashed in a mortar, and particles passing through a sieve having forty meshes to the centimeter collected. Five hundred milligrams of this sifted material were fed in 100 grams of meat to a kitten without producing any ill effect. This amount of orpiment is slightly in excess of the largest amount of "arsenic" which an "arsenic eater" has been reported to have eaten (25). This experiment shows that it will require more than sixty-six or one hundred times the amount of this preparation of powdered orpiment to produce vomiting than it will of dissolved arsenic.

Significance of results

The preparations of arsenious oxide containing the larger particles are less toxic to rats, rabbits and chickens than those containing the smaller particles. Large particles, however, are relatively more toxic to chickens than to rats and rabbits. Since the chicken possesses its own grinding mill (gizzard) for comminuting food coarse preparations of "arsenic" are probably ground therein to some extent. This will account in one way for the much greater potency of large particles to chickens than to rats and rabbits. Moreover, the certain lethal dose of the preparation containing the larger particles usually produced death more slowly in rats and rabbits than the certain lethal dose of the preparations of the smaller particles or the dissolved oxide.

This points to the probability that the larger particles administered tended to remain longer in the gastro-intestinal canal, where they constituted a source of continuous poisoning. Small particles will of course be removed by the act of solution, whereas those of medium size can continue longer to exert their effect, unless they are removed by peristaltic action. It was also observed that very large particles will not adhere to mucous membrane as readily as those of medium size, and consequently would be removed by peristalsis more readily.

The ratios of emetic potency of the different preparations are somewhat wider than toxic (lethal dose) ratios for these same preparations. This can be partially explained in several ways. The full potency of dissolved arsenious oxide is obtainable at once, whereas that of the undissolved oxide is obtained only as it is dissolved. The period over which the administered "arsenic" is capable of producing an emetic effect is almost certainly shorter than that in which a fatal dose can be absorbed. When medium sized particles are not eliminated in the feces they constitute a source of late poisoning but not necessarily one of late vomiting.

In the experiments with solid arsenious oxide only the isometric crystalline form was used. This is also probably true for experiments of other investigators who refer only to the undissolved oxide. Different results, however, might be expected from the use of the true amorphous (glassy) and the monoclinic forms, but they need not be considered here because of their rarity and instability. While it is to be conceded that the "rate of solution" (9) is an important factor "in absorption," its influence cannot be estimated when only one preparation is used. The writer's experiments indicate, however, that this is by no means the only the only factor. The relation of other factors to the rate of solution will be discussed when the experiments are published in full.

Although it may be true that the chemical composition of the different preparations is the same, the experiments here reported indicate clearly that "arsenic" from the pharmacological point of view is not always the same "arsenic," as has been tacitly assumed by clinicians and pharmacologists.

II. HABITUATION AND CHRONIC INTOXICATION

If then it be admitted that it has been proved in the preceding part that the toxicity of different preparations of undissolved arsenious oxide may vary greatly according to their state of subdivision, it follows that the literature on the habituation to "arsenic" needs a critical re-examination and re-interpretation in the light of these new facts.

Such an analysis, introduced by a brief discussion of the scope and of the criteria of habituation, follows, together with an enumeration of phenomena with which the supposed habituation must not be confused.

Scope of "habituation"

According to Wells (35), habituation to many poisons of this class (in contradistinction to true toxins) is largely fictitious, since chronic poisoning occurs. The term "habituation" as applied to arsenious oxide is usually limited to the undissolved material because of the tacit assumption having been made that all preparations are equally toxic, and that they approach very nearly the toxicity of dissolved arsenious oxide. Therefore, a high degree of habituation was believed to be apparent for the undissolved form, which was clearly recognized as not being demonstrable for the dissolved material (4), (12).

Criteria of "habituation"

The ability of a man or an animal to withstand one or more lethal doses at frequent intervals has been taken as a standard for judging habituation (4), (12), (15). Although this criterion would be quite adequate, particularly if the habituation is not fictitious, nevertheless, sufficient evidence as to the amounts of the particular preparation of undissolved oxide constituting such a dose has never been presented. It is a curious fact that de Busscher (2), who is cited as an authority on the oral lethal dose (4), (10), (11), (12), (15), used an exceptionally potent preparation. If by chance he had used one which was relatively innocuous probably many conclusions would never have been

published as their absurdity would have been apparent. Some of the so-called habituation experiments have been interrupted by vomiting. As an amount of arsenious oxide smaller than the lethal dose causes vomiting, and as the oxide has been administered by feeding, it is evident that emetic reaction might have been taken as an additional criterion.

Although the writer has raised, for point of argument chiefly, the fallacy in the high degree of the habituation to undissolved "arsenic" believed to have been attained, the minor degree of tolerance (4) which clinicians and investigators believe they can obtain with the dissolved material would seem worthy of a thorough re-investigation. If the past impression that has been obtained from experiments is indicative of the maximum tolerance possible, obviously some very delicate criteria must be developed. These would necessitate, among other things, if gastro-intestinal reactions are used, the accurate determination of the initial and final sensitivity, the same physical condition of the subject throughout the experiment, and the adequate control of the amount and character of the stomach contents, with an even mixture of arsenious oxide therein.

Phenomena from which "habituation" must be distinguished

Although several phenomena may be closely associated with an habituation, in making an analysis it is advisable that these should be entirely separated. It is necessary, therefore, to consider the habituation as distinct from any individual resistance of "arsenic" eaters caused by diet, etc. (Cushny (5)), the possible therapeutic effect of the "arsenic," and the development of a chronic intoxication. The difference between the velocity of and the duration of the action of arsenious oxide administered in the dissolved and undissolved states, which was dwelt upon in the discussion of the writer's experiments, must also be considered.

No data upon the subject of the influence of the state of inflammation of the gastro-intestinal tract upon the absorbability of arsenious oxide were found by the writer. McCrudden (18) has shown that certain irritants decrease the excretion (secretion by some part of the gastro-intestinal system) of morphine

administered subcutaneously. It is well known that sodium fluorid, an irritant, depresses absorption by the intestine. While it would be impossible to predict how arsenious oxide would affect its own absorption, it is evident that the information will be incomplete until the effect of the type and degree of inflammation is ascertained.

"Arsenic" eaters

For many years clinicians and others have been interested in "arsenic" eating. Although the existence of "arsenic" eating was known to Schallgruber (26) as early as 1820, no serious attention was given to this matter until 1851 and 1853 when von Tschudi (31) (32) brought it again to the attention of the medical profession. At first this observation was vehemently denied by several toxicologists (34),⁶ Taylor (29) being among them. In later editions of his works, Taylor was forced to omit his denial (34), although he refused to concede the popular interpretation placed upon "arsenic" eating (30).⁷

The discussion (1) which occurred during the time that recognition of the reality of arsenic eating was becoming established, shows that the impunity with which certain people eat large amounts of "arsenic" was misinterpreted as signifying actual

⁶ MacLagan (20) sums up the polemics as follows: "But the averment 'that the story of the Styrian arsenic-eaters is not only unsupported by adequate testimony, but is inconsistent, improbable, and utterly incredible' (Kesteven, *Asn. Med. Journal*, 1856, p.811); or that these are 'absurd and exaggerated statements, utterly inconsistent with all that is known concerning the action of arsenic in this or other countries, and but for the fact that they for a time received the literary support of Professor Johnston, and were diffused by him in an amusing book, they would not have required any serious refutation' (Taylor, *On Poisons*, 2nd ed., p. 92); or that it is a 'mess of absurdity,' 'a pure fable' (Christison, *Edin. Med. Journal*, 1855-56, pp. 709-710) are, although justified by a state of knowledge at the time they were made, no longer tenable."

⁷ Taylor states ((30) p. 50-51) that "*Habit* appears to have so little influence on arsenic, under the most careful medicinal use of it in this country, that no medical practitioner has ever succeeded in causing a patient to take *two grains* at a dose, the smallest quantity yet known to have destroyed life." "But no scientific witness who has seen anything of the operation of arsenic can allow these statements" (i.e., about arsenic eaters) "to influence his opinion of its ordinary effects on human beings."

immunity.⁸ It never seems to have occurred to the opponents of this idea that the relative innocuousness of the particular "arsenic" might explain the observed facts, although the effect of the degree of fineness of a particular preparation upon the rate of solution was known (Taylor (28), p. 256). The fact that particles of white "arsenic" may persist in the stomach for many days or months after the death of an individual was also known (Taylor (28), pp. 295-296), as well as the fact that "arsenic" eaters consume the "arsenic" in the form of relatively large particles. In spite of the possible significance of these facts in suggesting an explanation, most of the opposition seems to have subsided as soon as chemical identity of the material consumed was shown (20).⁹

The evidence indicating that the "arsenic" is eaten in a relatively non-potent state is found in the following facts. *Hütterrauch* (furnace smoke) or "*hidri*" (16) (20) (26) (31), obtained from the chimneys of smelters (16), and undoubtedly consisting of sublimed arsenious oxide, is eaten in lumps (20) or small bits (31). Some people, however, use the natural mineral, orpiment (arsenious sulphide) (20) (25), which contains arsenious oxide as an impurity (20) (28). Others have crunched lumps of "arsenic" with their teeth (1) (20) (30), or have consumed it after it has been carefully ground to a powder (?) on a "clean piece of paper" (20). Some sprinkle it in powdered form (?) on food, while others are said to allow it to "dissolve" (?) in the mouth (32). Crystalline (sublimed) arsenious oxide, however, is exceedingly difficult to pulverize in a mortar, and the impracticability of using a "piece of paper" or the "teeth" for securing a very fine preparation is apparent. The rate of solution of crys-

⁸ Taylor also states ((29) p. 94), "Assuming that one-half of the statements is based on truth, they prove,—not that arsenic may be swallowed in very large and increasing doses as an effect of habit—for it is said that the same dose, (two or three grains,) was taken for many years,—but that there must be some national idiosyncrasy among the Styrians which renders them proof against the effects of poisonous doses of arsenic under certain circumstances."

⁹ Taylor ((29), p. 92) states that a Mr. Kesteven "has collected a number of facts from the arsenic-works of Cornwall, which clearly prove that the so-called arsenic or *hidri* of the Styrian peasants, cannot be arsenic as it is known in England."

tallized arsenious oxide is so slow, to say nothing of the insolubility of orpiment, that without unimpeachable proof no credence can be given to statements that the "arsenic" dissolves in the mouth, or to the *post hoc ergo propter hoc* inference that it dissolves to a significant extent in the stomach and intestine.

Schäfer (25) places the amount of undissolved arsenious oxide consumed by "arsenic" eaters at 2, $4\frac{1}{2}$, and $5\frac{1}{2}$ grains (0.13 to 0.35 gram). Ordinarily, if this were dissolved fairly rapidly and retained, it would probably constitute an oral lethal dose for a man. The actual toxicity of the preparations used, however, cannot be stated, since the degree of fineness is not known. The same may be said of the precipitated arsenious sulphide, which, in the same state of fineness has been found by the writer to be somewhat less potent than arsenious oxide.¹⁰

Analyses of three-month old unpreserved samples of urine obtained from "arsenic" eaters were reported by E. Buchner (17). Large proportions of sub-lethal doses are said to have been eliminated in the urine during the first twenty four hours or less. It would be interesting to have this type of an experiment repeated, using preparations of "arsenic" standardized on animals suitable fore- and after periods, feces analyses, etc. Heffter (14), however, has disputed the accuracy of Buchner's method and analytical data. E. Buchner's results, moreover, do not prove that these "arsenic" eaters were in the habit of subjecting themselves repeatedly to tests of this described severity or that all the "arsenic" believed to have been found in the urine came from that which was administered.

Von Tschudi (31) recognized the relativity of the immunity in reporting that one eater "died poisoned a very painful death," and that fatal cases were by no means inconsiderable. Parker (23) reported the case of a Canadian, but did not establish that death was due to arsenical poisoning.¹¹ Schäfer (24) also reported a case in which the dose was sufficient to produce vomiting on numerous occasions. The fact that so few reports state that harm has come to "arsenic" eaters as a result of their

¹⁰ Unpublished experiments of the author.

¹¹ Dr. Douglas Symmers kindly consented to review this case.

habit, together with the failure of H. Buchner (17) to find evidence of chronic intoxication in eight "arsenic" eaters, might be construed to indicate that tolerance develops. On the other hand, the scarcity of evidence of harm tends equally well to support the idea of relative innocuousness of the "arsenic" consumed, particularly since this conception is the only possibility having a proven rational experimental basis.

Significance of experimental work on animals

Since it was tacitly assumed that individuals acquire an habituation and since the character of this habituation was not examined experimentally in recent years until this doctrine that a true habituation occurs had become firmly fixed in men's minds, pharmacologists in studying this phenomenon did not raise the question whether or not a true habituation really is developed. This is the more strange as two pharmacologists (10), (27), though actually recognizing their failure to obtain habituation, have not questioned the doctrine of habituation to "arsenic." It is believed that the analysis of the experimental evidence, in the light of the observations of the writer, that different arsenious oxide preparations vary greatly in toxicity, proves that the whole structure of the theory of the habituation to "arsenic" rests upon a single experiment of Joachimoglu (15) which, when critically examined, seems inconclusive. If the main evidence that habituation to "arsenic" occurs consists of a single inconclusive experiment, and if the observations of the writer furnish the key to a simpler explanation of the phenomenon of "arsenic" eating, then it is believed the time has come to question seriously the validity of this dogma.

Danger and Flandin (6), (8) appear to have been the first to investigate habituation to "arsenic" experimentally. Their experiments, which were performed some time previous to 1841, do not appear to have been conducted in connection with any "arsenic" eating discussion. They reached the dose of 1 gram per day for a dog after nine months, while at other times dogs died from "fractionated" doses. During the life of the animals believed to have been habituated, arsenious oxide was found

only in the solid excrement, none having appeared in the urine. While their chemical procedures were not modern, the positive results of the chemical tests obtained on the viscera of animals succumbing to the experimental treatment, in contrast to the negative tests for arsenic on the animals which they killed, support the conclusion that the arsenious oxide which they successfully fed without poisoning animals, was not appreciably absorbed. Their observations relating to the so-called habituation, should be interpreted at present only on the basis of relative insolubility and innocuousness of their preparations, because they give no data on the character of their preparations of arsenious oxide.

Hausmann (10), (12) is the first investigator to treat the subject of habituation to "arsenic" experimentally, after the occurrence of the controversy on the question of "arsenic" eating. Curiously enough, he states that he was unable to demonstrate any significant habituation. This he assigned to the variation in the high natural resistance of his experimental animals (hens), since he determined for himself the oral lethal dose of his preparation of undissolved arsenious oxide. As a matter of fact he tacitly assumed, in speaking of the high resistance of the hen, that all preparations of the oxide possessed equal potency. The conclusion to be drawn from his data however, is that he was unable to demonstrate an habituation in hens when he knew the lethal dose of the preparation used. These experiments are extremely important, because Hausmann appears to be the first investigator to prove, even though unconsciously, the fallacy in the logic of the usual interpretation placed on "arsenic" eating.

A dog used by Hausmann (10) vomited on receiving 20 mgm. of arsenious oxide per kilo. By beginning with small doses, however, the dog was able to retain this amount without harm after six weeks of feeding, although 25 mgm. made it severely ill. Hausmann cites de Busscher's (2) lethal dose for dogs (15 to 20 mgm. per kilo) as proof of the immunity of this dog against undissolved arsenious oxide. It is quite evident from Hausmann's data on the emesis, however, that if an immunity were

acquired, it could not possibly be calculated to have been more than 25 per cent above the normal resistance. In view of the fact that nothing is known of the preparation used, the amount of food and the uniformity of the size of the particles administered from day to day, his conclusions cannot be accepted.

In another experiment reported by Hausmann (11), a dog weighing about 21 kilos finally took 1.2 gram of undissolved arsenious oxide twice a week. A slightly larger dose than this caused vomiting in this animal. As no data concerning the preparation used are given, the significance of this experiment is not apparent. De Busscher (2) is again cited as an authority on the oral lethal dose for dogs. In a subsequent discussion Hausmann (12) cites the experiments of Roussin, who used calcium arsenite, and states that the degree of habituation could not be estimated because the lethal dose of the calcium arsenite was not known. It is further evident, therefore, that although Hausmann appreciated the necessity of ascertaining the exact oral lethal dose of a substance used, toxicologists had not yet recognized the fallacy of the tacit assumption that different preparations of undissolved arsenious oxide always possess the same potency.

Cloetta (4) has reported what he believed to be the record degree of habituation. A dog weighing about 8 kilos finally received 414 mgm. of undissolved arsenious oxide per kilo. No reference is made as to the fineness of the arsenious oxide, the relative uniformity of the particles administered from day to day, or to the lethal dose of this particular preparation. The significance of this experiment, therefore, is not evident. Cloetta stated that the excretion of arsenic in the urine was always very small and that the immunity of the dog lay in its ability not to absorb the arsenious oxide. Subsequently this dog succumbed to the usual subcutaneous lethal dose of dissolved arsenious oxide for dogs. This experiment presumably shows (if a single experiment is indicative) that the "arsenic" fed either had not been appreciably absorbed, or if absorbed, had produced no systemic immunity.

Referring to Cloetta's habituation experiment, Green (9) infers that "many of the cases of high tolerance for arsenic are to

be attributed to very low absorption of coarse arsenious oxide from the intestine." He states further that "there is equally little doubt that nothing like so high a tolerance could ever be established for the easily soluble arsenites."

Cloetta's (4) dog had previously received, during the first part of the experiment, dissolved arsenious oxide. It was able to take a maximum daily dose of only 25 mgm. which, however, is much less than a lethal dose (2) (33) for a normal dog. If this dog received 500 grams of food, the concentration of arsenious oxide therein would be equal to the minimum emetic concentration (50 parts per million) herein reported for cats. Although different species, different animals of the same species, and, from time to time, the same animal, may vary slightly in sensitivity, the exactness of this apparent coincidence is indicative that the real relation may not be far removed. It is possible, moreover, that the maximum range of concentrations which an investigator has at his command in studying the emesis may be too small to be of use in attempting to prove slight differences between normal animals and those believed to be habituated.

Cloetta's (4) experimental data on the effect of dissolved arsenious oxide appear to need much further elucidation before they can be accepted as indicating in the slightest degree any tolerance. Animals (dogs and rabbits) fed increasing doses of dissolved arsenious oxide often died with intestinal disturbances, although this investigator believed that by beginning with small doses he was able to avoid this in some instances. Since the rabbits received their arsenious oxide in their drinking water, the question naturally arises if a water-free diet is sufficient to prove that none of the arsenious oxide was lost by spilling of the drinking water. One rabbit died after the 34-mgm. dose, believed to have been daily consumed in the drinking water, was increased to 36 mgm. and the administration took place by stomach tube in half doses, morning and evening. Another animal is stated to have run a similar course. If these experiments indicate anything positive, they presumably show that the rabbits were not consuming all the arsenious oxide put into the drinking water. A third rabbit (weight not given), stated to

have received 33 mgm. daily in its drinking water, was given 14 mgm. of arsenious oxide by stomach tube twice daily on three consecutive days. This animal was killed for chemical analysis. Since an amount constituting a lethal dose and the time of death vary somewhat in experiments with dissolved arsenious oxide, the untimely sacrifice of this animal renders the interpretation of this experiment in doubt. Throughout all of Cloetta's experiments there is in a few instances only the slightest presumptive evidence that tolerance was obtained. This contrasts so markedly with his admitted failures that the writer raises the issue that tolerance even to dissolved "arsenic" is not proved.

Joachimoglu (15) is one of the most recent investigators to report experiments on the habituation of higher animals to arsenious oxide. His experiments differ, however, from those of Cloetta (4) and of Hausmann (10) (11) on the dog, in that he performed one experiment on the acute toxicity (lethal dose) of the undissolved arsenious oxide. It might be presumed, therefore, that he fulfilled the requirement of standardizing his preparation (if it is possible to do this with a single experiment). This single experiment is the one previously mentioned in this paper as inconclusive, and upon which, it was stated, the whole support of the habituation to "arsenic" dogma rests. It is evident, however, since he cites de Busscher (2) as an authority on the lethal dose of undissolved arsenious oxide, which dose is not necessarily the same for all preparations, that he himself did not appreciate the fact that the toxicity is markedly influenced by the state of fineness of the preparation administered. It therefore follows that it should not be assumed by readers of his article that he unconsciously always fulfilled the requirement of having his preparation uniform throughout his experiments, or his dogs believed to be habituated consumed doses of the particular preparation ordinarily lethal to dogs.

A definite interpretation of Joachimoglu's (15) chemical data upon the absorption and the urinary excretion is not possible for two reasons: first the size of the particles administered from day to day may have varied; second, it is impossible to predict that particles (particularly of certain sizes) will be eliminated from the

bowel in a regular and orderly manner, and that some will not adhere for a longer time than others to the mucous membrane of the gastro-intestinal tract. The variations he obtained in the relative absorption as the dose was increased would seem to be explained better by the first possibility although the effect of the second cannot be positively ruled out. Joachimoglu's data show, however, that as the dose increased the net amount absorbed somewhat increased, and the animals developed chronic intoxication. Qualitatively, this is only what might be expected of a non-immune animal. The questions then arise, in view of the observed tendency for the absorption to increase, Can any real virtue be possessed by undissolved arsenious oxide which is not also possessed by the dissolved? Why have investigators preferred the solid form?

A clue to the solution of these questions, yet unanswered by the followers of this faith, is found in several places, one of which is in Joachimoglu's (15) own data. A dog, believed to be habituated, which received daily 25 mgm. of undissolved arsenious oxide per kilo, succumbed to a single dose of 15 mgm. per kilo of dissolved arsenious oxide (as a sodium salt). This experiment presumably shows, as was claimed, that the gastro-intestinal tract was not impervious to dissolved arsenious oxide. It should also be recalled that Cloetta (4) believed that his dog possessed no systemic immunity and stated that the habituation was due to lack of absorption of the undissolved arsenious oxide administered. Just why the gastro-intestinal tract should have been relatively immune to the undissolved oxide, which must dissolve, if one is going to consider an habituation, was never answered by these investigators.

A key to the solution of this question is found in the experiments of the writer, in which he recovered, practically intact, in the feces of cats, crystals of arsenious oxide and of orpiment which had been administered in the food. It follows then that undissolved arsenious oxide and that orpiment should be expected to produce an effect only to the extent that they dissolve. Casual consideration, therefore, should vindicate the suitability of the dissolved oxide for studies on habituation. Since the

undissolved oxide has always been relied upon, apparently because large amounts could be consumed only by using this form, a suspicion is cast upon the theoretical possibility of habituation of higher animals to "arsenic" that perhaps after all it is insusceptible of proof.

O'Kane, Hadley, and Osgood (22) fed arsenious oxide, probably in the solid state, to guinea-pigs for forty days or less. About 2 or 2.5 mgm. of arsenious oxide per animal was the lowest daily dose which, in some instances, produced death. This daily dose, however, is much less than the single fatal dose of dissolved arsenious oxide.

Sollmann (27), who studied chronic intoxication to arsenious oxide in rats, found that his results contrasted markedly with the beneficial effect popularly assigned to small doses of "arsenic." The largest harmful dose (averaging 0.0049 mgm. per day) reported by Sollmann is about $\frac{1}{10,000}$ of the minimum lethal dose for rats of the most potent preparations of undissolved arsenious oxide used by the writer.

The negative results of Brouardel (3), as well as those of Morishma (21) upon attempted habituation to dissolved arsenious oxide are also of some interest. Morishma states that he injected small doses in seven rabbits, at intervals of five to ten days, for four months, and at the time of writing that he had lost four animals.

The analysis of the literature just given shows the lack of conclusive evidence that any noteworthy degree of habituation to dissolved arsenious oxide has ever been proved. It is also more than probable that the impunity with which apparently large doses of undissolved arsenious oxide have been administered *per os* is to be attributed to the administration of the oxide in the form of relatively coarse and insoluble particles.

SUMMARY AND CONCLUSIONS

Experiments upon rats, rabbits and chickens have shown that the toxicity of different preparations of arsenious oxide administered undissolved *per os* varies markedly owing to the coarseness

or fineness of their subdivision. The finer the particles of a preparation, the more potent it is and vice versa. For the mammals used (rats and rabbits), the preparations consisting of the larger particles, when given in suitable amounts, produce death more slowly than those consisting of smaller ones. Preparations consisting of large particles are relatively more toxic for chickens than for these mammals. Experiments upon cats have shown that potency of arsenious oxide (administered undissolved) to cause emesis also depends upon the state of subdivision of the preparation of arsenious oxide used.

No generally valid statement as to the size of the exact lethal dose of undissolved arsenious oxide when administered orally can yet be given. The toxicity of each preparation must be determined experimentally, although it may be possible to estimate it approximately by determining the fineness of the "arsenic" with a low power microscope. It is unsafe to judge this point by the naked eye because a preparation which appears fine to the naked eye may consist of particles so large as to possess relatively slight potency. In the light of the facts presented in this paper, therefore, the mere consumption with impunity of large doses of undissolved arsenious oxide is not proof that habituation exists. The toleration of such large doses may be due not to any attribute inherent in the consumer (i.e., an immunity), but to an attribute inherent in the preparation of arsenious oxide consumed, namely, its relatively slow solubility, which has been further decreased due to its coarse state of subdivision. In order to establish in a given case without chemical data the fact that habituation (fictitious or real) has developed, it is therefore necessary to proceed in the following way. Determine the oral lethal dose of the particular (identical) preparation of arsenious oxide used and report the precautions taken in determining the same, and then demonstrate that the animal supposed to have been habituated is able to tolerate either more than this dose or more than the lethal dose of dissolved arsenious oxide. If this can be demonstrated, the conclusion that habituation (fictitious or real) has developed would be justified.

In none of the reports of experiments with undissolved arsenious oxide which state that habituation occurs, have the requirements just outlined been fulfilled. They can all be explained as reasonably by assuming that preparations of arsenious oxide of variable and relatively low toxicity were employed as by assuming that habituation had occurred. This is the more probable as no investigator has yet been able to demonstrate conclusively the development of any certain degree of habituation to dissolved arsenious oxide. It is the contention of the writer, however, only that habituation of higher animals has not been proved, and not that it cannot exist. In the absence of positive proof, however, and in the face of certain negative results obtained by others, it can be said that the doctrine of habituation to "arsenic" is open to very serious question.

There are now under way in this laboratory quantitative experiments pertaining to chronic arsenical intoxication and habituation. In conclusion, I desire to thank my colleagues, particularly Dr. C. L. Alsberg for their valuable criticism of this manuscript.

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A CHEMICAL METHOD OF ASSAYING THE ACTIVE PRINCIPLES OF DIGITALIS¹

ARTHUR KNUDSON AND MELVIN DRESBACH

From the Laboratories of Biochemistry and Physiology, Union University Medical Department, Albany Medical College, Albany

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Digitalis assay for many years has been a question of much debate. It has generally been conceded that the biological methods are more reliable than the chemical methods for determining the activity of digitalis. The literature bearing upon the physiological standardization of digitalis and its allies is very extensive and for a review the reader is referred to the articles by Hamilton (1), Rowe (2), Roth (3), Richaud (4), and Eggleston (5).

Most of the chemical methods which have been developed for digitalis assay have been based on separating and estimating the amount of one of the glucosides, particularly digitoxin, and judging from the amount of this principle the potency of the preparation. No single glucoside, of course, can be used as a basis for estimating the activity as there are at least three different active digitalis principles known which differ considerably in their physiological activity and, therefore, must be taken into consideration in any chemical method of assay.

Only two chemical methods, as far as the writers are aware, have been reported which claim to give results comparable to the biological assay. The first of these was developed by Martindale (6) and is based on a colorimetric reaction of the active constituents with Froehde's reagent—sulphuric ammonium molybdate. The process consists, briefly, of mixing 10 cc. of the tincture with 10 cc. of water, precipitating with lead acetate,

¹ A report of this work was presented before the American Society for Pharmacology and Experimental Therapeutics, in New Haven, December, 1921.

removing the excess of lead, filtering and evaporating to dryness. The residue is carefully extracted with chloroform, the chloroform solution evaporated, the residue taken up with warm water, filtered, evaporated to dryness, extracted again with cold chloroform, evaporated and the residue dissolved in 4 cc. of glacial acetic acid. 0.1 cc. of this solution is mixed with 1 cc. of sulphuric ammonium molybdate reagent in a test tube and the color which develops after standing five minutes is compared with a standard scale. In this way he determines whether a tincture is above, equal to, or below standard so that the method estimates approximate activity only. Three hours are required to carry out the test.

The second chemical method is that of Beery (7), based on the lines of Martindale's method, but with essential differences as to extraction, precipitation and "working up" of the resulting glucosidal residues. He has devised two colorimetric processes called A and B. A is concerned with the water soluble glucosides and B with total glucosides. In process A the digitoxin and saponin are eliminated and the colorimetric estimation gives the therapeutic value. In process B the colorimetric value for total glucosides is obtained. The colorimetric value for B-A gives the toxic value of the digitalis tincture and the ratio $\frac{B-A}{A}$

permits judging of the tincture. Both of these methods require considerable time for completion and are very cumbersome compared to the biological methods. They give results which only approximate the strength of the preparation and it has not been shown by either of the investigators that the color reaction varies in accordance with the physiological activity of the glucosides.

In 1918, Baljet (8) reported upon a new identity reaction of digitalis glucosides. The reagent consisted of equal parts of a 1 per cent solution of picric acid in 95 per cent alcohol, plus a 10 per cent solution of sodium hydroxide. In the presence of a cardiotonic glucoside, whether from digitalis or strophanthus, etc., an orange red color is imparted to a few drops of the reagent, the color intensity attaining a maximum in about twenty to thirty minutes. He found that the reaction was positive with digitoxin,

gitalin, g-strophanthin, k-strophanthin and negative with digitonin, arbutin, amygdalin and conduragin. The test was found to be very sensitive and gave a reaction with at least 0.1 mgm. amorphous gitalin, 0.05 mgm. crystalline digitoxin and 0.03 mgm. strophanthin Merck. Baljet believes that the lactone function in the molecule of the glucoside is essential in producing the test. Wischo (9) does not believe the reaction can be applied to galenical preparations for he states that the digitalis glucosides react with different intensities of color and that the strophanthins show the same intensity of color despite differences of toxicity. If we wished to determine the quantitative amounts of glucosides present his view would be correct, but Wischo did not take into consideration that the color intensity might vary with the physiological activity. Baljet observed, however, that the glucosides react with increasing intensity of color in the order of their physiological activity, and our results, as will be shown below, indicate that the intensity of the color reaction bears a direct relation to the physiological activity of the active principles of digitalis.

METHOD

Principle

The method here proposed is simple and based upon the Baljet reaction given above. It consists of decolorizing the digitalis preparation with lead acetate, removing the excess lead and then treating the decolorized solution with an alkaline picrate solution and allowing the color to develop. The intensity of the color, which is measured in a colorimeter against a standard, is found to vary with the physiological activity and gives results which are in close agreement with those obtained by the bioassay of Hatcher and Brody (10).

Solutions needed

1. A 10 per cent solution of neutral lead acetate; 2.5 cc. are used for each determination.
2. A 10 per cent solution of Na_2HPO_4 ; 1.25 cc. are used for each determination.

3. Alkaline picrate solution. This solution is prepared by mixing 95 cc. of 1 per cent purified picric acid solution with 5 cc. of 10 per cent hydroxide solution. The picric acid and sodium hydroxide solutions can be made up as stock solutions and the alkaline picrate solution freshly prepared as needed. Five cubic centimeters of this solution are used for each determination.

4. As a standard for comparison a solution of crystalline ouabain containing 0.266 mgm. in 5 cc. of water is prepared. Five cubic centimeters of this solution added to 5 cc. of the alkaline picrate solution give a color equal in intensity to that of 0.5 cc. of a standard tincture assayed by Hatcher cat method when treated in similar manner and diluted to 10 cc. volume with water. In other words, the color of the standard is considered equal to half a cat unit as expressed by Hatcher's method. This standard solution put in a pyrex flask has kept for at least a month without showing any deterioration. Some standard solutions tested have kept for almost a year and others have lost about 10 per cent of their activity after standing more than a month so that at present we make up a fresh standard each month.

5. A permanent standard has been prepared by dissolving 3.44 grams of pure potassium bichromate in water and making up to a litre. The color of this solution set at 20 mm. in a colorimeter is equal to the color given by 5 cc. of standard ouabain solution when mixed with 5 cc. of the alkaline picrate. The permanent standard is not quite as good a match with the color from digitalis preparations as the ouabain standard but good results can be obtained with it.

Procedure

Five cubic centimeters of a tincture of digitalis, or an equivalent amount of drug in the form of a fluid extract or infusion,² etc., are measured into a 25 cc. volumetric flask and diluted with water to about 15 cc. To this solution 2.5 cc. of the lead acetate are added, contents mixed, and then diluted with water to the

² In case the amount of tincture or other solution being tested is limited, as little as 2 cc. can be used and corresponding amounts of precipitating reagents and dilutions then made.

mark. After mixing thoroughly and allowing to stand a minute the solution is filtered; 12.5 cc. of the filtrate are then measured into another 25 cc. volumetric flask and 1.25 cc. of Na_2HPO_4 solution are added in order to precipitate the excess lead. The contents of the flask are then diluted to the mark, mixed thoroughly, and filtered. The filtrate should be crystal clear but may have a slight tinge of yellow color.

Transfer 5 cc. of the filtrate to a 10 cc. volumetric flask or tube graduated at 10 cc. and at the same time transfer 5 cc. of the standard ouabain solution to another volumetric flask. To both of these flasks add 5 cc. of the alkaline picrate solution, mix, and allow to stand at least twenty minutes; make color comparison in the colorimeter, setting the standard most conveniently at 20 mm. The color comparison should be read between twenty and thirty-five minutes after the alkaline picrate has been added so that it is never advisable to develop the color in more than three to five specimens at a time. Instead of using the standard ouabain solution the permanent standard of potassium dichromate solution mentioned above can be put in the colorimeter, set at 20 mm. and the unknown specimens compared after color has been allowed to develop in the manner described.

Calculation

The depth of the unknown (in mm.) divided by the reading of the standard, and multiplied by two times the number of milligrams of drug in the aliquot portions of specimen tested gives the number of milligrams of drug equivalent to a cat unit as expressed by the Hatcher and Brody method. If, for example, 5 cc. of a decolorized filtrate of a tincture are used, the equivalent of 0.5 cc. of tincture is 50 mgm. of drug, and if the reading of the tincture is 17.2 against the standard set at 20 mm., then the formula will work out as follows:

$$\frac{17.2}{20} \times 2 \times 50 = 86.5 \text{ mgm. to 1 cat unit.}$$

Or, take another example: if 0.25 mgm. of digitoxin are used and

the reading of the digitoxin is 16 against the standard at 20 mm., then the formula will work out

$$\frac{16}{20} \times 2 \times 0.25 = 0.40 \text{ mgm. to 1 cat unit.}$$

Notes on the method

The quantity of tincture taken for the determination is based on the average value for a standard tincture and variations in the amount of tincture or other preparations used may be necessary in special cases. In testing the powdered leaf a 5 per cent infusion can be conveniently made and 10 cc. of this infusion used for the test. For tests on purified preparations of digitalis and the active principles such amounts are taken for the test as would be equivalent in activity to 5 cc. of tincture. Also, in the case of the pure principles of digitalis the preliminary procedure of decolorization can be omitted, although the treatment with lead acetate and sodium phosphate does not remove any of the pure principles from solution, as shown by Martindale (6).

In carrying out the procedure described above, care must be taken not to have the solution of active principles and alkaline picrate solution contain more than 3 or 4 per cent of alcohol, as larger amounts of alcohol will markedly effect the reading; this is due to the fact that alcohol imparts a darker color to the alkaline picrate solution. The amount of alkali added to the picric acid in preparing the alkaline picrate must be accurately measured as larger or smaller amounts increase or decrease the intensity of color.

If the reading of the unknown against the standard set at 20 mm. is greater than 30 mm. or less than 10 mm., it is well to repeat the determination, adjusting the quantity of the unknown so that it will read about the same as the standard.

The colors are developed at room temperature and we have found that the ordinary range of temperature in a laboratory, from 15°C. to 25°C. does not have any appreciable effect on the reaction.

REMARKS ON THE CAT METHOD

The results that have been obtained by the above described method have been checked by the Hatcher and Brody (10) cat method of digitalis assay. In carrying out this method, we have not departed in any essential details from their procedure. Food was withheld from the cats for twenty-four hours. They were etherized and weighed to within ten grams. The femoral vein was exposed and connected by a glass cannula to a 50-cc. graduated burette containing the solution to be tested. (This operation ordinarily takes from three to five minutes.) Throughout the injection, the animal was very lightly etherized, that is, just sufficiently to prevent struggling. The uniformity of the anesthesia was indicated by the respiratory rate and depth. We have used both the simple and "combined ouabain" methods of Hatcher, that is, we have injected a given specimen slowly until death of the animal, usually in about ninety minutes, and in other instances we have given about one-third of the estimated fatal dose of a specimen in ten minutes' time and, after an interval of twenty minutes, finished with a 1:100,000 ouabain solution in 0.9 per cent NaCl in approximately one hour. This procedure is believed to be of value when one is dealing with slowly acting glucosides (10). In the latter method the calculation of the activity of the specimen may be simply carried out as follows: Tinctures are diluted 1:10 with a 0.9 per cent sodium chloride solution and fluid extracts diluted to 1:100. Suppose the cat's weight is 2.86 kgm. and that, after injecting 10.6 cc. of a diluted tincture, 17.5 cc. of a 1:100,000 ouabain solution (i.e., 0.175 mgm.) are required to kill. As 0.1 mgm. of ouabain per kilogram is a fatal dose, i.e., one cat unit (10), 0.175 mgm. equals 1.75 cat units. Therefore, 2.86 minus 1.75 equals 1.11 cat units, satisfied by the 10.6 cc. of diluted digitalis tincture injected at first. As the 10.6 cc. represents 1.06 cc. of the original tincture, then $1.11:1::1.06:X$, and X equals 0.955 cc., or one cat unit. If we assume that 1 cc. of the tincture represents 100 mgm. of dry leaf, then in the present case 95.5 mgm. of digitalis equal one cat unit. In other words, the tincture is somewhat stronger

than a standard tincture in which 100 mgm., on an average, are regarded as equal to one cat unit.

As Eggleston (5) has discussed the advantages and disadvantages of the cat method, as compared with others, it will suffice to consider a few outstanding points:

1. Evidence that death is due to the action of the glucosides on the cardiac mechanism: As is well known, the action of these bodies is not confined absolutely to the heart. Nevertheless, we do not agree at all with Hamilton (11) that death is almost invariably caused by paralysis of the respiratory center. Eckler's (12) observations on sixty-nine cats confirm Hatcher's contention that in the cat method the end point is cardiac and not respiratory failure. Eggleston's paper (5) continues the argument in favor of Hatcher's conclusion, which is further supported by the electrocardiograph studies of Robinson and Wilson (13) and Levine (14). In fact, the electrocardiograph demonstrates the very early and continuous action of these substances on the heart. In our own research, we have seen the respiration fail before the heartbeat ceased in only three or four cats in one hundred and sixty, and in these few cases too much ether was given. In all but five or six instances, we have examined the heart as soon as respiration ceased, and, with six exceptions, have found the left ventricle in a contracted state, the right being dilated to a greater or lesser degree. Often, the left chamber has the firmness of a muscle in extreme rigor; in a few cats, it had a medium hardness and was sometimes seen beating feebly or showing fibrillation. However, the presence of weak beats, even if coördinated, would not necessarily mean that respiration failed first. In the six exceptions both ventricles were well dilated, though these cats, barring one, were not deeply etherized. It was noted that five cats showing a dilated left ventricle were rather slow to succumb. According to Sollmann (15), diastolic arrest in the intact mammalian heart is due to low coronary pressure preventing the heart muscle from contracting. This is doubtless true in prolonged experiments especially, and in cases in which the arterial pressure falls rather early as a result of the presence of digitonin, or similar substance, in the solution. If, as with most

of our cats, the animal is poisoned quickly with the glucosides in question, that is, within ninety minutes or so, experiments show that a good arterial pressure exists until near the end, providing, of course, that too much of some anesthetic has not been given. Finally, one must not forget the possibility of accidental coronary obstruction in the injection experiments.

2. The variability of cats and the number required in digitalis assay. Although there is no doubt that cats react to digitalis and allied principles in a variable way, and in a manner which is sometimes disappointing, the necessity of eliminating the absorption factor was imperative in our work. Furthermore, the action of these glucosides on the cat's heart resembles more closely their action on the human heart, as Eggleston (5) has shown. We have, in most instances, used three or more cats in an assay, and have been satisfied with a result when the maximum variation from the average in a set was not over 30 per cent; generally, it was not over 25 per cent and often was only 5 to 6 per cent. In our tables given below, the number of cats in a given assay is usually the number left after excluding those giving results deviating more than 30 per cent from the average of the set. In this way we follow the common practice in biological assay work. In our experience we have encountered resistant and susceptible cats more frequently than would be expected from the figures cited by Eggleston (5). However, our series is not so extensive (it includes two hundred individual animals at this writing). We estimate that at least 5 per cent were atypical in reaction. Though such individuals must be excluded from a series, they suggest problems of great interest, problems which are met with in the clinical use of digitalis and which will still be encountered in spite of improvements in the types of digitalis preparations administered.

3. The question of anesthesia. Except in individual instances, it would seem that it makes no difference whether the cat is anesthetized or not during injection. Ether suffices for the purpose and the anesthesia should be light. As the digitalis intoxication progresses, the depth of the anesthesia can be altered, as, for instance, in the later stages in which the blood pressure falls.

The ether can then be reduced or withdrawn altogether. If no anesthetic is used after the initial operation, the animal is likely to struggle violently. It soon tires of this in most cases, but, although there is no pain resulting from the injection, the cat may keep up a disagreeable crying. Possibly, violent exertions are as big a factor as the anesthesia in some cases because the heart rate is tremendously increased and the blood pressure raised. It is conceivable that the heart is put at a disadvantage thereby. Moreover, if one wants to study the heart sounds, as we did, at frequent intervals, the cat must be quiet.

4. Body temperature. Even in light anesthesia, the body temperature of the cat will fall considerably in the course of an hour or two. It is doubtful if the drop of two, or even three, degrees is of any importance, but it is well to protect the animal against unnecessary heat loss.

5. Rate of injection. For most digitalis bodies, this factor is not of great importance in the Hatcher method, for by taking advantage of the synergistic effects of ouabain, in the ninety-minute method, good results can be obtained in spite of the varying rapidity of action of the different glucosides. Levine (14), too, has shown that the action of ouabain is independent of the concentration in the blood ("speed of administration"). However, Hatcher (16) and Dooley (17) have recently pointed out that the rapidity of elimination of certain digitalis bodies may be of great moment, for they give good evidence that a digitalis body exists which has a cardiac action and which is very rapidly eliminated. The injection rate in the presence of such a rapidly eliminated fraction would be important, because with a slow rate of injection the readings would run too high.

6. Observations of the heart sounds and pulse rate. We have made it a rule to listen to the heart sounds and make note at short intervals of the changes in their character. We also set down changes in the heart rate and keep record of the rate of injection. Levine (14) has shown by the help of the electrocardiograph that signs of toxic action of ouabain appear in cats in the form of ventricular premature beats when, on the average, about 50 per cent of the lethal dose has been given. By auscultation

tion, we found that in the majority of thirty cats, upon which careful observations of the heart sounds were made, evidence of beginning digitalis action was noted with an average of 62 per cent of the fatal dose. The lowest in this series of thirty cats was 32 per cent and the highest 70 per cent of the fatal dose. In a rough way, the first evidence of action of the drug is an index to the toxicity of the specimen for a given cat. Aside from this aspect of the experiment, the changes in the heart sounds are very interesting and instructive.

RESULTS AND DISCUSSIONS

In order to determine whether the decolorizing process removed any of the active principles several preparations were assayed by

TABLE 1

Bioassay of digitalis preparations before and after decolorization

SPECIMEN NUMBER	KIND OF PREPARATION	ASSAY BEFORE	NUMBER OF DETER- MINATIONS	ASSAY AFTER	NUMBER OF DETER- MINATIONS
		<i>mgm. \approx c. u.</i>		<i>mgm. \approx c. u.</i>	
2	Tincture.....	84	7	83	4
15	Tincture.....	73	4	85	4
32	Tincture.....	29	5	30	2
18	Fluid extract.....	64	7	67	—

the Hatcher method before and after decolorizing and the results are given in table 1. The agreement between the two is very close so that it does not seem as though any of the active principles were removed by the decolorizing process.

In table 2 are given the results of comparative tests on a series of tinctures, fluid extracts, infusions, and one purified commercial preparation. These preparations represent specimens from most of the large pharmaceutical manufacturers and, as may be noted, vary in strength considerably, emphasizing the need of assay. In the last two columns are given the values in average activity per cent by the two methods. The average activity expresses the percentage of the theoretical activity as calculated from the amounts of standardized crude drug employed or supposedly

TABLE 2
Assay of tinctures, fluid extracts and infusions of digitalis

SPECI- MEN NUM- BER	KIND OF PREPARATION	CHEMICAL ASSAY	BIOLOGICAL		AVERAGE ACTIVITY PER CENT*	
			Assay	Num- ber of deter- mina- tions	By chemi- cal assay	By biologi- cal assay
		<i>mgm. \approx c. u.</i>	<i>mgm. \approx c. u.</i>			
1	Tincture.....	98.0	93.0	4	102.0	107.0
3	Tincture.....	77.0	73.0	3	130.0	137.0
3	Tincture (diluted 1:10 and tested 10 months later).....	76.0	77.0	3	132.0	130.0
5	Tincture.....	86.0	92.0	3	116.0	109.0
9	Tincture fat free.....	127.0	157.0	2	79.0	63.0
14	Tincture.....	115.0	101.0	3	87.0	99.0
15	Tincture.....	78.0	73.0	4	128.0	137.0
16	Tincture.....	60.0	61.0	4	167.0	164.0
23	Tincture.....	73.0	64.0	4	137.0	156.0
24	Tincture.....	96.0	90.0	5	104.0	111.0
25	Tincture.....	105.0	88.0	4	95.0	114.0
28	Tincture.....	68.0	59.0	3	147.0	169.0
30	Tincture.....	73.0	61.0	5	137.0	164.0
32	Tincture.....	62.0	29.0	6	105.0	224.0
4	Fluid extract.....	182.0	151.0	2	55.0	66.0
8	Fluid extract.....	84.0	67.0	4	119.0	149.0
18	Fluid extract.....	77.0	66.0	7	130.0	151.0
19	Fluid extract.....	90.0	85.0	3	111.0	118.0
20	Fluid extract.....	63.0	102.0	13	159.0	98†
21	Fluid extract.....	94.0	93.0	3	106.0	108.0
26	Fluid extract.....	101.0	118.0	3	99.0	85.0
27	Fluid extract.....	68.0	75.0	3	147.0	133.0
13	Infusion 5 per cent from bruised leaves.....	94.0	102.0	3	106.0	98.0
13	Infusion 5 per cent (10 months later).....	550.0	560.0	4	18.2	17.8
29	Infusion 1 per cent from powdered leaf.....	87.0	72.0	3	115.0	139.0
33	Infusion 2 per cent from powdered leaf.....	93.0	97.0	2	107.0	103.0
38	Digilusin.....	6.30	5.44	3	93.0	108.0

* Average activity per cent expresses the percentages of the theoretical activity as calculated from the amounts of standardized crude drug employed or supposedly employed.

† This is the only specimen which has given us any serious difficulty in getting a determination by the bioassay method and we have reported here the average for all of our thirteen determinations, as upon examination of the individual assays, as listed on page 217, we would be able to obtain several different averages.

employed. Upon examining these columns it will be noted that the two methods show an agreement within 30 per cent or less in twenty-three of the twenty-five different specimens tested and a majority of them do not vary by more than 10 to 15 per cent. With two of our specimens the results do not agree very well. Tincture 32 was assayed by Hatcher's method by the manufacturers and certified by them to be 65 mgm. equal to a cat unit. Our chemical assay agrees with their certified assay very closely but we are unable to satisfactorily explain the great discrepancy between our bioassay and theirs. We made seven determinations on this specimen and the lowest assay was 26 mgm. and highest 30 mgm. It may be possible that our group of cats at that time was especially sensitive or other biological or chemical factors may have entered into play. Unfortunately, we were unable to repeat these assays at another time as we sent the remainder of the specimen back to be re-assayed and have not received any report. Further work with similar preparations may clear up this marked discrepancy. Fluid extract no. 20 also shows a marked disagreement between the two methods. The result for our bioassay on this preparation is the average of thirteen determinations which do not by any means agree among themselves. The individual assays on this preparation in the order in which they were obtained run as follows: 134, 177, 111, 219, 106, 95, 35, 50, 24, 55, 41, 151, and 133 mgm., respectively. These determinations are, however, so variable that this preparation will require special study to see if we can determine the cause of this marked variation. With the exception of the above mentioned samples, the agreement between the two methods is remarkable and within the limits of error obtainable by any of the other biological methods.

Although we have compared all of our assays with the cat method, two of the preparations have also been assayed by the official frog method (18). Tincture no. 5 assayed by the frog method showed an activity of 120 per cent which agrees very closely with the chemical assay. Digiglusin (specimen no. 38), which is a purified commercial preparation containing glucosides of the leaf, is stated by the manufacturers to be seventeen times as

active as the drug. Their assay is carried out by the frog method and our chemical assay indicates an activity of sixteen times that of the drug.

The question might arise as to whether deterioration of specimens would be indicated by the chemical method. Two specimens were tested for this purpose, but one of them had not deteriorated as we had expected it to. Tincture no. 3 was diluted 1:10 with 0.9 per cent NaCl and allowed to stand in a cork stoppered bottle and after ten months had not changed in activity. Infusion no. 13 was allowed to stand in the laboratory in a glass stoppered bottle and after ten months it had decreased in activity to about one-sixth of its strength. The chemical method, as

TABLE 3
Assay of active principles of digitalis

SPECI- MEN NUM- BER	KIND OF PREPARATION	CHEMICAL ASSAY	BIOLOGICAL	
			Assay	Number of determi- nations
		<i>mgm. \approx c. u.</i>	<i>mgm. \approx c. u.</i>	
31	Digitoxin.....	0.40	0.30	4
37	Digitalin according to Kiliani.....	1.16	1.17	3
40	Digitalein according to Schmiedeberg	2.80	2.92	5

may be noted, agreed very closely with the biological, and, although we have only this one specimen showing marked deterioration, we believe that the chemical method would indicate any deterioration. Experiments along this line will be continued.

Comparative tests on the three glucosides that are believed to represent to a large extent the activity of digitalis are given in table 3. Digitalin was prepared by us from Merck's German digitalin by Kiliani's method (19) and the other two glucosides were the purest products we have been able to obtain. As to purity of these preparations we are not able to state; in fact there is some doubt as to whether any of the digitalis glucosides so far isolated can be considered chemical entities. However, the assays of these specimens by the two methods agree very well.

Digitoxin varies more than either of the other two but more determinations by the bioassay method might bring them closer together.

The application of Baljet's reaction and our method to other members of the digitalis series of drugs is being studied and we hope to report these soon in a separate communication. We are likewise obtaining more data for comparison between the chemical method and the biological assay methods of digitalis preparations.

CONCLUSIONS

Although the number of specimens that we have tested has been rather limited, due to the large number of animals required for checking by the bioassay method, we believe that the chemical method as outlined above presents several distinct advantages over the biological methods which at present are the only accepted ones. Briefly stated, they are:

1. The method is simple, and any one having a chemical training with an understanding of the use of a colorimeter should be able to carry out the procedure.

2. It renders the pharmacist independent of the trained physiologist or pharmacologist.

3. The reaction upon which the method is based is extremely sensitive, and 0.01 mgm. of digitoxin diluted to a volume of 10 cc. with alkaline picrate solution can be detected; in other words digitoxin can be detected in 1:1,000,000 dilution.

4. The time required for carrying out the test is shorter than with most of the biological assays. With the reagents on hand, an assay can be completed within an hour. In a series of three determinations run at one time we have completed them in an hour and a half.

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THE ŒDEMA OF PARA-PHENYLENEDIAMINE

O. S. GIBBS

Pharmacology Department, University of Edinburgh

Attention was originally directed to para-phenylenediamine in consequence of its poisonous effects when used as a hair-dye, or as a dye for furs.

These effects were usually irritation of the skin followed by eczema, and in some cases of hair-dye poisoning by a definite swelling of the eye-lids and face.

Dubois and Vignon (1), who first investigated its action in animals discovered that its administration to dogs caused, amongst other symptoms, an acute œdema of the head and neck, particularly in the orbital region, where marked exophthalmos and raised intraocular pressure were noted.

Puppe, (2) working with rabbits also found this marked œdema, but in these animals the tongue and neck tissues were more severely affected than the eyes. Other workers, Kunkel (3) Erdman and Vahlen, (4) Laborde and Meillère, (5) and Meissner (6) confirmed these results, and Meissner who is the latest author on the subject states that this œdema, as well as the other symptoms, can be entirely prevented in rabbits by repeated doses of atropine given subcutaneously.

An acute local œdema preventible by atropine seemed to invite further investigation, and I have accordingly carried out a series of experiments to determine the cause of the localization of the œdema, and whether atropine acted by its effects on the parasympathetic, or in some other way.

Preliminary experiments in rabbits amply confirmed that para-phenylenediamine produces an œdema of the head and neck, and that this is sufficient to cause the death of the animal by asphyxia due to an acute œdema of the glottis and vocal cords.

Rabbits poisoned by para-phenylenediamine show a most extraordinary and typical appearance. The face and neck are markedly swollen, in some cases the eyes bulge from their sockets, the mouth is wide open and the tongue protrudes, this, being due to a huge translucent blister-like swelling under it which lifts it up and forces it forwards out of the mouth. The swelling so fills the buccal cavity that the jaws cannot be closed. Incisions into this swelling or into the neck edema reveal a curious condition, for although watery fluid exudes from the cut tissues, they do not tend to collapse at all, and the wound gapes, giving an appearance like a cut into a ripe plum.

Dissection reveals the presence of further swellings, involving the epiglottis, pharynx and larynx, and also the pre-tracheal muscles. The salivary glands are not affected.

The most typical feature of all, however, is that presented by the vocal cords. These are swollen to the size of a small currant and present the appearance of two little cushions of clear fluid pressed together in the middle line and completely occluding the air passage. These swellings, like that under the tongue, do not collapse on incision, and one cannot withdraw more than a drop or two of fluid through a hollow needle.

The lungs are rarely oedematous, and oedema was not found in the axilla, abdominal wall, groin or legs, nor was any abnormal fluid present in the pleural or abdominal cavities, though some occurred in the pericardial sac.

These results were confirmed in cats, where the oedema is in similar places, but is much more sharply defined. The swelling under the tongue is particularly well marked and characteristic and is very readily controlled by observation; these animals were thus chosen for further experiments fig. 1.

Pure para-phenylenediamine is a crystalline body having a faint pink color; it is quite soluble in water, forming a pale pink solution, neutral to litmus. The drug was obtained from Kahlbaum.

The dose used in my experiments was 0.1 gram per kilo, when given by the stomach tube; for intravenous use, a dose was calculated to give approximately 1:1000 in the blood stream.

This latter being a slightly higher dose than that given per os. Doses were invariably weighed and dissolved just prior to administration.

With such a dose, given to cats or rabbits, either by stomach tube, subcutaneously, or intravenously, œdema commences to appear about one and one-quarter hours after the administration.



FIG. 1. PHOTOGRAPH FROM A TRACHEOTOMIZED CAT POISONED WITH PARA-PHENYLENEDIAMINE

In this case the orbital tissues are affected

This is first noticed under the tongue, but very soon after the neck is found to be swollen just below the jaw; along with the development of the visible œdema is a gradually increasing respiratory difficulty, an indication of swelling of the glottis and vocal cords. In some cases the eyes were affected, and it is interesting to note that the intraocular tension was raised, the bulb becoming very hard. Eventually the œdema becomes so

great, and especially that affecting the vocal cords, that the animal dies of asphyxia, usually three to four hours after the administration of the drug.

Meissner states that tracheotomy performed rapidly prevents the death of the animal for some hours. Animals on which tracheotomy was performed beforehand certainly do not die of asphyxia.

My first experiments were directed to the antagonism of atropine, and I found that 2 mgm. atropine sulphate hourly, given hypodermically, does not influence the development of the oedema at all, that these doses paralyze the parasympathetic nerves is shown by the failure of the vagus action on stimulating it, and by the dilatation of the pupil. Thus atropine in these doses has no effect antagonistic to para-phenylenediamine, and Meissner himself shows that in rabbits the preventive action is only obtained with very large doses 0.012 gram half-hourly) and suggests some specific action. It is doubtful if cats would tolerate such large doses. Cutting the vago-sympathetic on both sides has also no effect on the oedema.

Tying the carotids practically stopped the oedema, so that the reduced amount of blood supplied by anastomosis is insufficient to supply the fluid necessary for its production. Was this oedema then due to a local effect on the vessels either directly, or through the nerves? Removal of the sympathetic, or superior cervical ganglion produced no constant change in the production of the oedema; adrenalin proved to have its typical action, showing that the sympathetic was neither stimulated nor paralyzed. Meissner also gives experiments in which the sympathetic was cut and allowed to degenerate with the same negative results.

If one however stimulates the sympathetic on one side, the oedema formation of that side is greatly delayed (figs. 2 and 3). This result is practically constant, but the explanation is probably that on the stimulated side the vessels are contracted and so less blood goes to the part in a given time. That there is no true preventive effect is indicated by the final production of a maximum oedema, which merely takes a longer time to appear.

Motor and sensory nerves were cut and stimulated with no constant results. The possibility was considered that some action originating in the brain was transmitted by the sympathetic—such as blushing in the human species. I had it, however, on the authority of Professor Cushny that this œdema appears equally well in decerebrate animals. Cutting the spinal cord in the neck and destroying it up to about the third cervical vertebra did not prevent its appearance.



FIG. 2



FIG. 3

FIG. 2. SHOWS THE LOWER JAW OF A POISONED CAT FROM THE RIGHT SIDE

FIG. 3. SHOWS THE LEFT SIDE OF THE SAME CAT AS FIGURE 2

Illustrating the delay in the œdema production following continuous stimulation of the cervical sympathetic ganglion on that side.

These experiments gave no support to the view that the nervous system is concerned in the formation of the œdema.

Blood pressure tracings were taken, and one did not find any fall of pressure take place other than that which could be accounted for by the experimental procedure and the anesthetic. These experiments were prolonged until a typical œdema appeared, and although by no means conclusive, do not tend to

favor the view of a capillary poison. These experiments, however, drew attention to the accelerated clotting of the blood, which following the administration of para-phenylenediamine takes place so rapidly as to cause great difficulty in measuring the blood pressure. Meissner also draws attention to this point, and shows that it is associated with an increased fibrin content in the blood.

The coagulation time was measured by Dale and Laidlaw's method (7). The blood was obtained from the carotid artery by means of a short paraffined cannula ($\frac{1}{2}$ inch) which was carefully cleansed with plugs of dry cotton wool between each observation. Four observations were usually taken as a group and the figures given are an average of each group.

	TIME AFTER PARA-PHENYLENEDIAMINE				
	Normal	$\frac{1}{4}$ hour	$\frac{1}{2}$ hour	1 hour	$1\frac{1}{2}$ hours
	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
1	49.6	39.7	33.7	15.1	
2	34.3	12.6	9.4	8.1	Too fast to time

Interference in the lymphatic flow was looked for in experiments in which the lymph flow from the head was measured. This was carried out on the left side. The junction of the lymph ducts with the veins was dissected out; the veins on each side of the junction were tied leaving $\frac{1}{2}$ inch of the most convenient—sometimes the jugular, sometimes the left sub-clavian—on the side furthest away from the heart. A cannula was then inserted into the vein and several tiny veins having been ligated, the lymph runs quite clear or white if chylous. The thoracic duct was then ligated as also was the lymph branch from the upper extremity. The flow, which is very slow, was measured by a Condon drop recorder. This experiment is very difficult to carry out after para-phenylenediamine as the lymph then clots with great rapidity and blocks the cannula, and also the lower end of the duct, and it is often impossible to clear them. In one experiment the flow was measured continuously for four and one-half hours—until the animal died. There was a temporary

increase after the administration of para-phenylenediamine, followed by a more prolonged decrease, to be increased again just prior to death. Para-phenylenediamine was detected in the lymph. Conclusions could be drawn from these experiments that lymphatic blockage is not the important factor, since the amount of decrease measured would by no means account for the fluid of the œdema and secondly, although other lymphatics were ligatured, including the main duct, no unusual œdema was caused in other parts of the body.

A minute examination of the poisoned animals showed that the dorsum of the paws, particularly the forepaws, were constantly œdematous under para-phenylenediamine, as was also in one case the tail-base. These findings were confirmed in rabbits, where subcutaneous œdema also appeared in the flanks.

Thus it was clear that the œdema of para-phenylenediamine is not so narrowly localized as has been suggested, but is a general action extending over many tissues. It still remained a question why the effect is so much more marked in the head and neck, where the poison acts. Up to this point I had detected but one positive fact, namely, that some change had occurred in the blood, as shown by the increased rapidity of clotting; was the action of para-phenylenediamine in causing the edema then to be correlated with this, and was the point of action on the blood itself, and not on the vessels?

I perfused the systemic circulation of cats by means of a cannula placed in the ascending aorta, the fluid used being fresh aërated Ringer at 37° at a pressure of 1.8 metres. In three-quarters to one hour œdema appeared in the head and neck and was almost identical in position and extent with that produced by para-phenylenediamine (figs. 4 and 5). This also was present in the paws, and again more marked in the forepaws. Accompanying this œdema the intestine was filled with fluid as was also the bladder. This had also been noted in the para-phenylenediamine experiments, although the intestinal fluid was not in such quantity and was more limited to the stomach. If one continued this experiment eventually nearly all the tissues became sodden. This experiment showed quite clearly that if

a diffusible fluid is in the circulation it tends to pass out in larger quantities in the area in which the edema of para-phenylenediamine occurs. This probably depends on two factors: the amount of vascularization, and the looseness of the tissues, and a combination of these two factors would give the ideal condition for œdema production. That there is yet a third factor is evidenced by the fact that very occasionally œdema



FIG. 4



FIG. 5

FIG. 4. PHOTOGRAPH SHOWING THE RESULTS OF AORTIC PERFUSION WITH RINGER AT 37°C. FOR ONE AND ONE-HALF HOURS

FIG. 5. THE LOWER JAW OF THE SAME CAT AS FIGURE 4 DISSECTED TO SHOW THE TONGUE SWELLING

hardly develops in an animal poisoned by para-phenylenediamine, although the animal lives sufficient time for it to occur. In all such cases I found a particularly pronounced effect on the heart, and in all probability the failing heart was barely able to do more than just keep the animal alive; in other words, the blood pressure was at a minimum. Further, as is shown by the perfusion experiments, one does not get the characteristic sharply defined œdema unless the pressure is at least 1.8 m.

The third factor is thus not unlikely to be the pressure at which the fluid is circulating.

Experiments were repeated with 6 per cent gum Ringer solution at the same temperature and pressure. In these the oedema was not developed so quickly nor is it quite so typical, being more diffuse. The addition of 1:1000 para-phenylenediamine to gum-Ringer solution gives the same oedema as the gum-Ringer alone. Frogs' vessels perfused also show no change in the rate of flow after para-phenylenediamine.

The cause of death in my anaesthetized and tracheotomized animals was not due to asphyxia as in the normal animals, but apparently to heart failure. This appears to be due to a direct action on the heart, for in most cases the left ventricle was partially or completely in systole, and in a few cases the right ventricle also showed this condition. Frog's hearts perfused with 1:1000 para-phenylenediamine in Ringer stopped in systole in twenty minutes: with weaker solutions this may take much longer.

Para-phenylenediamine is excreted in all body fluids tested which included the urine, stomach and intestinal contents, lymph, pericardial fluid, fluid in the mouth and bronchi. The test used was purely empirical, since it was found that the ordinary test of ferric chloride and hydrogen sulphide is not sufficiently sensitive in body fluids. This test consisted of adding clean, dry, pine sawdust to the fluid when in a short time a bright red color is developed in the sawdust. It is surmised that it may depend on a furfurol reaction. This test is sensitive to 1:5000 in water and is given readily by para-phenylenediamine in the serum or in urine. No normal fluids give this reaction. Meissner, using the ferric chloride test, states that para-phenylenediamine is not excreted unchanged in the urine, as Puppe had previously stated.

Microscopic examination of the blood showed the cells to be normal. Quantitive estimations were not carried out. The spectrum of the blood was also quite normal.

DISCUSSION

In investigating the cause of any form of œdema the chief factors to be borne in mind are changes in the nervous system, the vessels, and the blood.

My experiments show that the œdema of para-phenylenediamine is not directly influenced by any nervous action, though this may be of secondary importance by regulating the amount of blood to a given part.

Attempts to show any alteration in the vessels gave negative results, the blood-pressure being well maintained and the surviving vessels showing no distinct change in calibre when perfused with the drug. The change in the heart may give rise to suspicion that the vessel walls are also affected, but on the other hand this may be explained by the œdema of the myocardium which Kunkel and Puppe have described, so that the cardiac action may be associated with the result, and not the cause of the œdema.

There is some evidence of a change in the blood in the diminution of the coagulation time, and this suggests that the blood may be the point of action of para-phenylenediamine. For example any change causing an aggregation of the colloids, and so producing a more diffusible liquid, would favor the escape of fluid through the vessel walls. This view though largely theoretical is not entirely without basis, since a diffusible liquid such as Ringer gives an almost identical form of œdema; but the question whether the edema arises from some such change in the blood, or from some obscure change in the vessels cannot be finally decided at present. There can be no doubt, however, that it is not localized in certain tissues, but is of a general nature. The lungs seem to be less affected than the general tissues, for pulmonary edema does not occur under para-phenylenediamine, and thus presents some difficulty whatever the explanation of the general edema is accepted. It is possible however that the lower pressure in the pulmonary vessels may be inadequate to force the lymph through the walls, for even in the systemic circulation the edema is prevented by a restricted circulation through the tissues; this is shown by compressing the vessels or constricting them by stimulation of the vaso-constrictor nerves.

SUMMARY

1. Para-phenylenediamine produces a typical œdema of the head and neck, which is however not confined to them, but is the initial stage of general œdema.

2. Atropine in doses sufficient to paralyze the parasympathetic nerves does not antagonize the action of para-phenylenediamine.

3. The nervous system influences the œdema production only indirectly by changing the blood supply.

4. Para-phenylenediamine has an action on the blood as is shown by the shortening of the clotting time.

5. The œdema of para-phenylenediamine is closely imitated by that induced by the perfusion of an animal with Ringer's solution, showing that the action of the drug is a general one either on the blood-vessels or on the blood.

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CUMULATIVE ACTION OF COBRA VENOM

ARTHUR R. CUSHNY

Pharmacological Department, University of Edinburgh

In our work on cobra venom done some years ago Yagi and I (1) found great difficulty in immunizing rabbits and circumstances prevented us following out the cause. More recently I have made a number of experiments, and find that the venom presents a cumulative action similar to that seen under digitalis and its allies and also under such inorganic poisons as mercury, arsenic, lead, etc., under prolonged exposure to them.

I cannot find that Calmette (2) draws attention to this phenomenon in his description of the immunization against cobra venom, in which of course his object was to avoid such cumulation. The method (3) he recommends to obtain immunity however suggests that he was not without experience of it. He injected subcutaneously one-half the M.L.D. and then after twelve to fifteen days if the animal had regained its weight, again one half the M.L.D. Six days later 1 M.L.D. could be given, then again after six days 1.5 M.L.D. and after the same interval 2 M.L.D.

Fraser (4) does not make mention of the difficulties in immunisation, though the tradition of the laboratory runs that these were considerable. His injections were also mainly subcutaneous though sometimes intravenous. Yagi and I always gave our injections intravenously.

Others who have worked with snake-venom have no doubt experienced the same difficulties as we met. Professor C. J. Martin informs me that he found it necessary to space his injections very widely.

In my experiments I have used a dried cobra-venom supplied to Sir Thomas Fraser in 1895 and kept in the laboratory since that time. A solution in Ringer's solution was made up fresh each day and injected within one hour at longest. The solution

generally contained either 0.5 or 0.2 mgm. per cubic centimeter. The rabbits were of medium size, 1500 to 2000 grams and were accurately weighed and the dose calculated per kilogram. The amount injected was generally less than 1 cc. and never more than 1.5 cc. The injection was always made into one or other of the ear veins: in the beginning of an experiment the marginal vein was used, but very often this gradually became obliterated from phlebitis and another had to be chosen. This obliteration proved a difficulty, but by beginning at the periphery and approaching the base of the ear in successive injections, it could be overcome. The phlebitis is apparently the result of some local action on the vein from the venom, for I have not seen it arise from the repeated injection of other poisons.

The minimal lethal dose was found by a series of injections.

Four rabbits received 0.3 mgm. per kilogram; all died.

Five rabbits received 0.25 mgm. per kilogram; 4 died, one was very severely poisoned but recovered.

Thirteen rabbits received 0.2 mgm. per kilogram; 12 survived and one died after two days (infection?).

Sixteen rabbits received 0.15 mgm. per kilogram; none presented any symptoms.

No obvious symptoms were observed in the great majority of those animals that survived. In two of the twelve which survived after 0.2 mgm. per kilogram, there was slight weakness in the course of two to three hours, but it could only be made out when the animals were made to run about, when they seemed more readily exhausted than normal ones.

The M.L.D. for this sample of cobra venom is therefore sharply marked at 0.25 mgm. per kilogram, while 0.2 mgm. per kilogram had no effect in the great majority of rabbits; in other words, a concentration in the tissues of 1 in 5 million was harmless, while 1 in 4 millions was fatal. Conversely, when an animal dies of cobra venom, its tissues must contain at least this latter concentration. The amount of venom remaining in the tissues in an active form after a given time can therefore be ascertained by finding the smallest quantity of venom which is now necessary

to kill and subtracting it from 0.25 mgm. This method has been used by Hatcher in work on the cardiac glucosides with good results.

The amount of venom of the first injection which has been destroyed, excreted, or neutralized,¹ may similarly be ascertained by subtracting 0.25 mgm. from the sum of the two injections. As regards the neutralization by antitoxin, I can find no evidence that this occurred in any significant degree in my experiments; the M.L.D. of the second injection was always below that necessary in the normal animal.

In Hatcher's experiments the second injection was made slowly and the amount which had been given when death occurred was noted. Under cobra venom, however, this method is inadmissible as the symptoms set in even more slowly than under the cardiac glucosides. It was therefore necessary to inject a single dose at the usual rate (about one minute) and to wait for the issue within the next twenty-four hours.

The results in the first series of experiments are given in table 1, in which the first column gives the number of the rabbit the second gives a series of days, one to twelve, on which the injections were made, the first being made on the first day, the second after an interval varying from one day to twelve. The dose is also indicated here in milligram per kilogram. The third column gives the results (death +, recovery 0); death in each fatal case occurred within sixteen hours of the second injection.

The remarkable result appears from this table that when 0.2 mgm. per kilogram is injected (80 per cent of the M.L.D.) it is not all got rid of in twelve days, and when 0.15 mgm. is injected (60 per cent of the M.L.D.) some of it persists in the tissues for at least six days. Calculation shows that of the 0.15 mgm. of the first injection in this case 0.1 mgm. or 66 per cent must have remained active for a week. On the other hand, in the experiments in which the total of the two injections was exactly the M.L.D., no fatality occurred, showing that some of the poison

¹ To avoid repetition "discharged" will be used in this paper to indicate venom that has been injected but cannot be traced further owing to its having been excreted or having been rendered inactive in some way.

TABLE 1

NUMBER	DAYS OF INJECTION AND AMOUNT INJECTED (MILLIGRAMS PER KILOGRAM)												RESULT	TOTAL INJECTED	EXCESS OVER M. L. D.	VENOM DISCHARGED		VENOM REMAINING	
																Amount	Days	Amount	Days
	1	2	3	4	5	6	7	8	9	10	11	12							
22	0.2	0.2											+	0.4	0.15	<0.15	1	>0.05	1
25	0.2	0.2											+	0.4	0.15	<0.15	1	>0.05	1
26	0.2			0.2									+	0.4	0.15	<0.15	3	>0.05	3
28	0.2			0.2									+	0.4	0.15	<0.15	3	>0.05	3
29	0.2					0.2							+	0.4	0.15	<0.15	5	>0.05	5
30	0.2						0.2						+	0.4	0.15	<0.15	6	>0.05	6
31	0.2										0.2		+	0.4	0.15	<0.15	11	>0.05	11
32	0.15	0.1											0	0.25	0.0	Trace	1	(?)	
34	0.15		0.1										0	0.25	0.0	Trace	2	(?)	
33	0.15	0.15											+	0.3	0.05	<0.05	1	>0.1	1
35	0.15						0.15						+	0.3	0.05	<0.05	6	>0.1	6
36	0.15					0.1							0	0.25	0.0	Trace	6	?	

was discharged in the interval before the second injection. This holds even when this interval was only 24 hours. There must therefore be a rapid "discharge" at first, which is not maintained later.

In order to ascertain how far the process of discharge continues later, a much longer interval between the injections was allowed (table 2).

Here an interval of twenty-eight to thirty days elapsed between the injections and it was found that in the second injection 0.15 mgm. per kilogram was ineffective, while 0.2 mgm. was fatal in three out of four experiments. The amount of the first injection still persisting in the tissues must therefore have been more than

TABLE 2

NUM- BER	1	28	29	30	31	RESULT	TOTAL INJECTED	EXCESS OVER M. L. D.	VENOM DISCHARGED		VENOM REMAINING	
									Amount	Days	Amount	Days
									mgm.		mgm.	
43	0.15	0.15				0	0.3	0.05	>0.05	28	(?)	
44	0.15	0.15				0	0.3	0.05	>0.05	28	(?)	
45	0.15	0.2				0	0.35	0.1	>0.1	28	(?)	
46	0.15	0.2				+	0.35	0.1	<0.1	28	0.05	28
47	0.15				0.2	+	0.35	0.1	<0.1	31	0.05	31
48	0.15				0.2	+	0.35	0.1	<0.1	31	0.05	31

0.05 mgm. (33 per cent) and less than 0.1 mgm. (66 per cent), and from the fact that one rabbit of the four injected with 0.2 survived, I judge that the former figure is the nearer.

At any rate the discharge in the later stages must have been minute for at least 33 per cent of the amount first injected persisted in the tissues for four weeks.

The question arises whether in some of these experiments a condition of anaphylaxis was not present and was the cause of death, or at any rate a contributing factor. This seems excluded however by the minute quantity of the initial injection (0.1 to 0.2 mgm. per kilogram) and further by the fact that the symptoms did not follow immediately on the second injection, but generally set in after thirty to ninety minutes, and were those induced by cobra-venom and not in any way similar to those of anaphylaxis.

The small difference between the harmless and the final fatal dose in table 2 also seems to be in favor of the toxic action rather than of anaphylaxis. These comparatively large doses thus proved fatal even when widely spaced, the amount discharged being only a small fraction of the first injection even after several weeks.

In another series of experiments smaller doses were given at comparatively short intervals (table 3). In the first two of these nos. 37 and 38 no better results were obtained, the rabbits dying from 0.3 mgm. divided into three doses and again failing to discharge more than 0.05 mgm. even in a week (no. 38). When the dose was reduced still further to 0.05 mgm. per kilogram, i.e., one-fifth of the fatal dose at intervals of two or three days, considerably over the M.L.D. could be injected in course of time, without the onset of symptoms (nos. 39, 40 and 51).

Nos. 49 and 50 show the cumulative effects of even these small doses when repeated at short intervals. In no. 49, 0.05 mgm. was given seven times in succession, the animal thus receiving 0.35 mgm. in eight days without any symptoms being elicited; in order to test if the poison was accumulating in the tissues, 0.1 mgm. was then given and proved fatal within four hours. No. 50 (see fig. 1) was treated in exactly the same way except that the dose which proved fatal to no. 49 was not given, the usual 0.05 being injected instead; this caused severe poisoning from which the animal had recovered next day. After an interval of one day, 0.05 again caused severe symptoms. The treatment was now omitted for four days and was then recommenced with daily injections of 0.05 mgm.; after four of these there was again a violent attack of poisoning with recovery. In this instance the rate of discharge of the venom may be calculated fairly closely: for the M.L.D. being 0.25 mgm. per kilogram and 0.2 mgm. not causing symptoms, the concentration in the blood on the occasions on which symptoms were presented must have lain between that caused by 0.2 and 0.25 mgm. injected intravenously—say 0.225 mgm. This was reached after eight injections of 0.05 mgm. in nine days—that is 0.4 mgm. was injected and 0.175 mgm. was discharged in 9 days or 0.022 in each of the

TABLE 3

NUMBER	DAYS AND AMOUNTS OF INJECTION																			RESULT	TOTAL INFECTED
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
37	0.1		0.1		0.1															+	0.3
38	0.1			0.1				0.1												+	0.3
40	0.05		0.05		0.05			0.05		0.05		0.05								0	0.25
39	0.05		0.05					0.05		0.05		0.05			0.05	0.05				0	0.4
51	0.05		0.05					0.05	0.05	0.05	0.05	0.05								0	0.5
49	0.05	0.05	0.05	0.05	0.05	0.05		0.05	0.1											+	0.45
50	0.05	0.05	0.05	0.05	0.05	0.05		0.05	0.05*		0.05*				0.05	0.05	0.05*	0.05*		0	0.65
55	0.15	0.05	0.05	0.05	0.05			0.05	0.05	0.05	0.07									+	0.35
56	0.15	0.05			0.05			0.05	0.05	0.05	0.05									0	0.47
57	0.15	0.05			0.05			0.05	0.05	0.05	0.05									+	0.45

* Indicates symptoms of poisoning following the injection.

eight intervals between the first and the eighth injection. In the next forty-eight hours 0.044 mgm. would be discharged, but a new injection of 0.05 renewed the concentration and the intoxication followed. The next interval of five days allowed the discharge of 0.11 mgm., so that before the injection on the sixteenth day the blood contained about 0.11 mgm. In the following four injections 0.2 mgm. was injected, but in the three

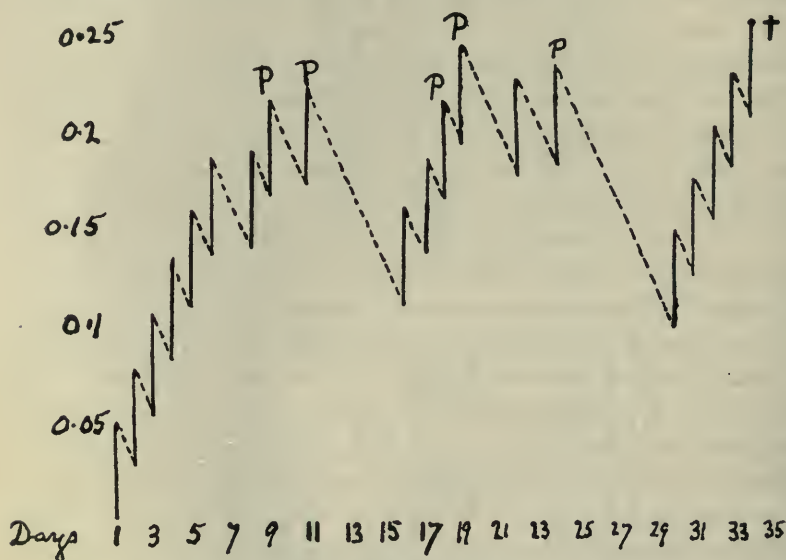


FIG. 1. GRAPH OF CALCULATED CONTENT OF VENOM IN TISSUES OF RABBIT 50

The unbroken vertical lines represent the amounts injected (0.05 mgm. per kilogram), the broken lines the calculated quantities "discharged" (0.022 mgm. per kilogram). *P* indicates symptoms of poisoning, which set in, whenever the concentration (calculated) exceeded 0.2 per kilogram. +, Death, when 0.25 was exceeded.

intervals 0.066 was discharged, leaving a total concentration at the end of the injection on the eighteenth day of about 0.22 mgm. which induced slight symptoms: a further injection on the nineteenth day raising the concentration to about 24 mgm. induced a very severe attack. The further course of this animal may be seen in the graph in which it is shown that two injections at intervals were made and then a longer pause allowed the concentration to fall to about 0.1 mgm. Five further injections

of 0.05 mgm. increased the concentration to above 0.25 which proved fatal. A certain latitude must of course be claimed for these calculations, but they show that of 0.05 mgm. injected, rather less than one-half was rendered innocuous or excreted in the course of 24 hours by this rabbit.

Nos. 55 and 57 show other examples of the cumulative effect of repeated small doses. In these the treatment was initiated with a larger injection of 0.15 mgm. in order to shorten the course of the experiment. In no. 56, the same treatment as in no. 57 was not fatal, and there may be variations in the ability of individual animals to get rid of the poison. This animal was kept apart for eleven days after the last injection and was then given 0.1 mgm. venom on each of two successive days and died with typical symptoms: this shows that while the small doses given previously had not accumulated in the tissues in sufficient strength to be fatal, some remained even after eleven days, and reinforced by the new injection, proved fatal.

Under treatment with digitalis the cumulative action is striking, not only from the occurrence of symptoms from a dose which has been repeatedly given without apparent effect, but from the suddenness of the onset. This was even more marked under the venom. Careful examination of the animals failed to detect any abnormality in behavior, but a single minute dose (0.05 mgm.) was sufficient to induce very serious or fatal symptoms in animals which had been treated with the same dose for some time previously.

One of the most marked symptoms under venom poisoning is the apparent exhaustion of the animals, which arises from the partial paralysis of the nerve ends and which is first manifested by a tendency to hang the head and to allow the fore limbs to slip forward. In most experiments in which I expected symptoms to arise soon, I tested whether the animals were more susceptible to fatigue by making them run about their cages actively in the expectation that they would show some exhaustion. It is easy to induce such exhaustion in hutch rabbits, but these cumulative rabbits were not more susceptible than control animals. Yet next day a single injection of 0.05 mgm. was often

sufficient to cause marked exhaustion or death, while in the controls it was without effect. The suddenness of the onset of symptoms is then due to the sharp line of demarcation between the innocuous and the fatal dose.

In experiments nos. 48-57 there is evidence that the animals were able to dispose of part of the venom injected and in no. 50 this has been calculated to amount to 0.022 mgm. or nearly half of each injection. In other experiments, e.g., no. 38, the amount of poison got rid of in the same time was much smaller. Thus nos. 49 and 50 received 0.35 mgm. per kilogram in eight days without harm, while no. 38 died from 0.3 mgm. in the same time. And in some of the experiments given in table 2, the whole of the first injection would have been disposed of before the second was given, if the discharge had been as rapid as in no. 49.

This indicates that while the discharge is fairly rapid at first, it becomes slower later and the last traces of poison are eliminated with great difficulty. This may be interpreted as indicating that part of the venom is free in the tissues at first and is therefore readily eliminated, while part is in some form of combination (e.g., adsorption) and is therefore retained. A parallel may be drawn with other drugs with which the body is flooded suddenly; thus when a large dose of morphine or iron is injected intravenously; it appears in the urine for the first hour or so, but then disappears from it though it is still present in the tissues in large amount. But this view is not justified unless the combination is admittedly of the lowest possible nature; for the same rapid initial elimination and prolonged retention is met with under such drugs as the bromides, which are apparently not combined in any way in the tissues, but circulate freely in the blood. Yet bromide may be found in the urine for a month after a single therapeutic dose. The prolonged retention of cobra-venom in the tissues does not necessarily imply any actual chemical or physical combination between them, though this is rendered probable by the experiments with curara to be detailed later in this paper. And in the same way the retention of the digitalis glucosides may arise not from their forming any combination with the tissues such as is often suggested; it is true that

there are other arguments in favour of actual combination in the case of the digitalis bodies such as the failure of all attempts to extract them from the tissues.

My experience is that if venom is given in small and frequently repeated doses, less is retained than when larger quantities are given at longer intervals, but this is compatible with either view, for it may be given as evidence of the slow formation of a bond with the tissues, or for the rapid elimination of the poison when its concentration in the blood is high and its slower disappearance as the concentration falls. In the case of the bromides, each new dose is followed by a rapid excretion, but the total in the tissues slowly rises just as in the cumulation of venom.

In these experiments, there is no evidence of either tolerance or immunity. Thus in no. 50, four injections of 0.05 mgm. on the sixteenth to nineteenth day caused severe poisoning, and five injections of 0.05 mgm. on the thirtieth to thirty-fourth day proved fatal. The failure to obtain the immunity of Calmette and Fraser is doubtless owing to the short duration of my experiments, but indicates the great difficulty of immunizing rabbits to cobra-venom compared with that met in experiments with such toxins as that of castor oil bean. The combination of cumulative action and tolerance has been described by v. Lhota in the case of the digitalis bodies, but his experiments extended over many months.

Death from cobra-venom in small doses occurs from paralysis of the terminations of the motor nerves such as is more familiar in curara poisoning, and the action was the same in cumulative experiments. But cumulation may occur without any appearance of weakness until the last fatal dose is given. It was therefore desirable to know whether the nerve ends were injured by venom before any obvious symptoms were present. For this purpose I compared the poisonous dose of curarine in normal rabbits with that necessary in rabbits treated with repeated doses of cobra-venom, but showing no symptoms of its action. A solution of curarine was prepared from the bark of *Strychnos toxifera* and partially purified. Injected into the marginal ear vein in

normal rabbits, it was fatal in doses of 0.04 cc. per kilogram, while 0.035 cc. produced severe symptoms, but these lasted only a few minutes and the animal returned to its normal state in half an hour. After 0.025 cc. per kilogram no symptoms appeared or only very slight and insignificant ones, which passed off in a few minutes. When a fatal dose of curarine was injected, the rabbits died within five minutes from failure of the respiration, while if they survived an injection for ten minutes, they recovered.²

Two rabbits (nos. 64 and 65) were now treated with intravenous injections of cobra-venom for three days, the successive doses being 0.15, 0.05, 0.05 mgm. per kilogram. They showed no symptoms whatever and even after some exertion on the third day showed no more exhaustion than control animals. On the fourth day, 0.025 cc. per kilogram of curarine solution was injected into one and killed it within three minutes; the other received 0.015 cc. of the same solution per kilogram and died within four minutes. At this stage of venom poisoning curara was fatal in doses of 62.5 and 37.5 per cent of the M.L.D. for normal animals.

The natural interpretation of these results is that in the cumulation of cobra-venom, the poison is stored in the motor nerve ends but does not impair their conduction enough to give rise to actual symptoms. Even after exertion there remains enough conductive material in reserve to carry the nerve impulses, and exhaustion still arises from fatigue of the nerve centres and not from that of the nerve ends. The curarine action is similar to that of cobra-venom and when it is superposed on it the animal dies from a comparatively small dose. In the same way a further small quantity of cobra-venom in chronic poisoning is sufficient to kill even if it is far below the M.L.D. for the normal animal.

An alternative view would be that the venom is not actually anchored in the nerve ends, but that its effects depend wholly on the concentration which exists in the fluids surrounding them.

² In the literature some cases are given in which men are stated to have died several days after wounds from arrows poisoned with curara; but in these the curara was obviously not the cause of death, but infection of the wound.

I do not think it has been actually stated that in the cumulation of the digitalis series the glucosides are not stored in the heart muscle, but this seems the logical sequence of Straub's view that strophanthin is not taken up by the myocardium in higher concentration than exists in the fluids used to perfuse it.

To determine this point two rabbits were treated with venom in the same way as the last, each receiving daily injections of 0.15, 0.05, 0.05, 0.05 mgm. of venom on four successive days. On the fifth day they were both normal; under light ether anesthesia one was bled from the carotid artery directly into the jugular vein of a smaller rabbit under ether. The weight of the smaller rabbit was found to be increased by 40 grams. After fifteen minutes, the latter received 0.03 cc. per kilogram of curarine solution (= 75 per cent of the fatal dose), which did not prove fatal. When a second injection of 0.02 cc. per kilogram of curarine solution was made eighteen minutes after the first, the respiration failed in two minutes. Seventy-five per cent of the M.L.D. was not sufficient to kill, but an additional 50 per cent after eighteen minutes was fatal. Considering the speed with which curarine is eliminated it seems likely that there was hardly more than the M.L.D. in the tissues at the moment of death. The transfusion of the blood of a venom rabbit thus did not affect the toxicity of curara in any way.

The other venom rabbit was similarly bled into a small rabbit whose weight rose 50 grams and this transfused rabbit then received an intravenous injection of 0.032 cc. per kilogram curarine solution (80 per cent of the M.L.D.). It died in ten minutes but the symptoms were not those of curara but were probably due to an embolus; besides death from curarine always occurs within five minutes of the injection in my experience. So that in this experiment again the previous transfusion of the blood of a venom poisoned animal did not seem to alter the sensitiveness to curara.

These experiments thus lend no support to what may be regarded as a humoral view of cumulation and therefore strengthen the more natural belief that during cumulation the venom is anchored to the nerve ends or receptors in striated muscle.

CONCLUSIONS

Cobra venom injected intravenously is cumulative in action in rabbits inasmuch as small doses, even one-fifth of the minimal lethal dose, given repeatedly prove fatal.

This appears to be due to the slow inactivation or elimination of the poison which seems to vary somewhat in different individuals. Evidence of the persistence of the poison in the tissues could be obtained in some cases for a month after the injection. This gives rise to no symptoms, but the animal succumbs to a sub-minimal additional dose of the venom or of curarine, which acts at the same point. This seems to indicate that the venom is held in the nerve ends or receptors, and transfusion experiments showed that it is not contained in the blood.

A small part of the venom injected is eliminated or inactivated and this seems to occur soon after the injection, while later the quantity in the tissues is reduced only gradually. This may perhaps arise from the combination with the tissues occurring only slowly, and thus a certain amount of venom escaping before it is anchored.

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STUDIES ON THE ACTION OF BARIUM

WILLIAM SALANT AND NATHANIEL KLEITMAN

*From the Department of Physiology and Pharmacology of the University of Georgia,
Augusta, Georgia*

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Considerable evidence has been accumulated indicating that barium stimulates every form of muscle. According to Wertheimer and Boulet (1) its action on the heart resembles that of digitalis. Filippi (2) corroborated these results and laid even greater emphasis than the previous investigators on the digitalis effect of barium on the heart. More recently Poulsson (3), Werschinin (4), and Trendelenburg (5) reinvestigated the subject and came to similar conclusions, but they strongly emphasized the difference in the reaction of the heart upon the internal and external application of barium. Its action on smooth muscle likewise received considerable attention. Dixon and Halliburton (6) and later Cow (7) have shown that the pressor effects produced when the soluble salts of barium are introduced into the circulation are due to contraction of the muscular substance of the arterioles. Baehr and Pick (8) demonstrated that the same effect is produced by barium on the vessels of the lung, and in a later communication (9) they have reported that it exerts the same action on bronchial muscle. Trendelenburg (10) and Titone (11) obtained the same results in their observations on the effect of barium on bronchial muscle. The studies on the action of barium on smooth muscle were later extended by Lieb and McWhorter (12) who used the gall bladder as a test object, and by Berezin (13) who made observations on its effect on the veins of the liver. These reports are in harmony with those previously reported on the same subject. Studies on the reaction of voluntary muscle to barium have shown that its action was similar to that produced on other forms of muscle. Experiments on the

voluntary muscle of the frog reported by Tournade and Marchand (14) indicated that barium was without any effect on curarized muscle, or muscle in which degeneration of the motor nerve endings occurred.

In the course of different investigations carried out at various times for a number of years, one of us (S.) observed on several occasions that the action said to be characteristic of barium was in some cases absent. It was deemed advisable, therefore, to make a systematic series of tests on the action of the metal under different conditions, the present report embodying the results of such an experimental inquiry. The effects of barium were studied on the isolated organs in different functional states, and especially after they have been previously subjected to the action of substances which produced well marked changes in their activity. Thus the action of barium in different concentrations, avoiding, however, strong solutions, was studied in the isolated heart of the frog and the turtle which were perfused with Ringer's solution alone for several minutes. This was repeated in experiments in which the action of barium was tested after previous treatment with mercuric chloride, aconitine, or cocaine. Further, in experiments on the isolated intestine observations were made with barium after previous treatment with aconitine. In the studies conducted on the isolated heart of the frog and of the turtle the same methods were employed as in previous investigations reported by us (15).

REACTION OF THE FRESH HEART TO BARIUM

The frog heart

The variation in behavior exhibited by the hearts of different animals after they are removed from the body and perfused with Ringer's solution presented an opportunity for determining whether the functional state exerted any influence on the reaction to barium. That the functional condition is an important factor in modifying the action of barium was very clearly shown in experiments on the frog heart (fig. 1). When a heart that was contracting vigorously and with normal frequency was subjected

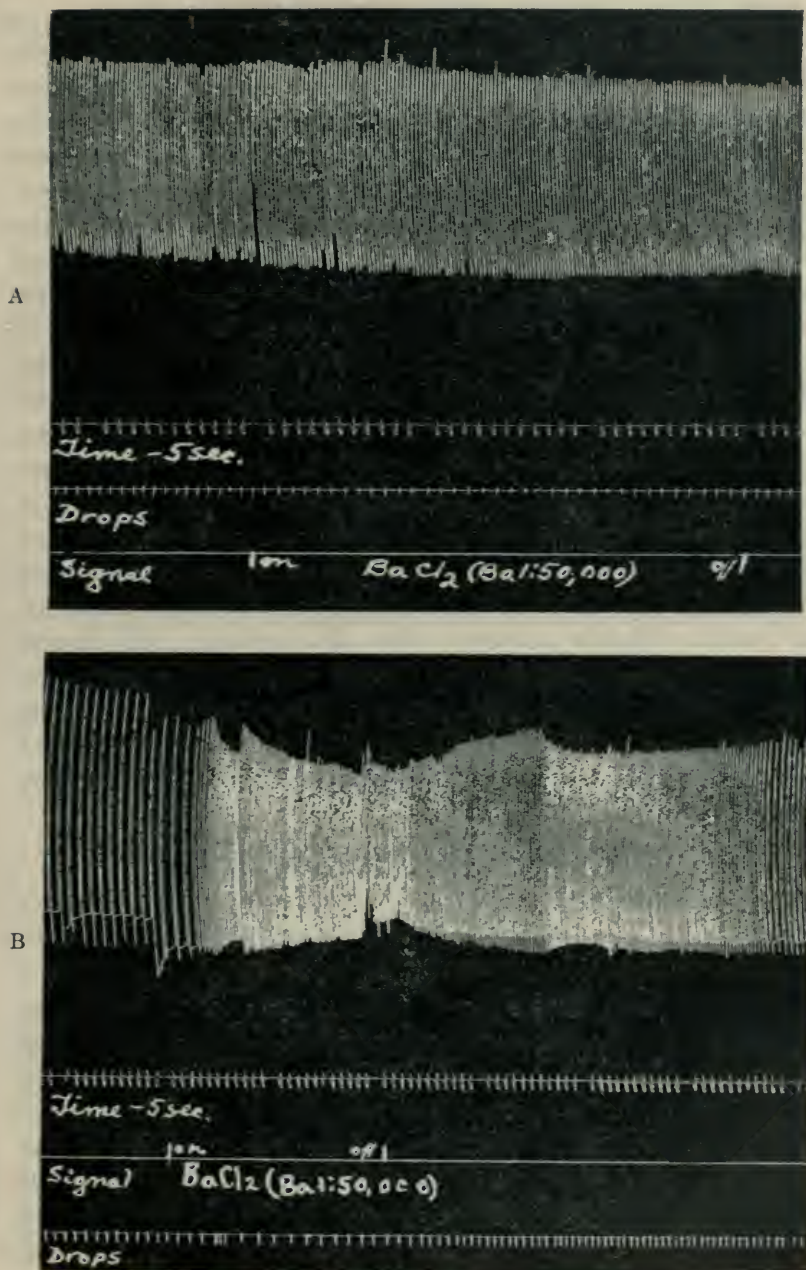


FIG. 1. FRESH UNTREATED FROG HEARTS PERFUSED WITH RINGER'S SOLUTION ALONE, THEN WITH RINGER'S SOLUTION CONTAINING BARIUM CHLORIDE

A shows that barium chloride had no effect when heart action was normal. B shows marked acceleration produced by barium chloride when heart was slow. Reduced one-half.

to the action of different concentrations of barium chloride, no marked changes could be observed, though the perfusion often lasted several minutes. The only effect noticed under these conditions was a moderate increase in frequency, and occasionally also some diminution in amplitude. Tonus may be stimulated slightly, or not at all. The effect was quite different, however, if the perfusion with barium chloride was carried out when the heart was slow or its action irregular. In some cases the fresh heart when perfused with Ringer's solution immediately after removal from the body either failed to react for some time, or would execute a few beats and then stop, while in others heart action was very slow, though the individual contractions were of good quality. Barium chloride (Ba 1:10,000 to 1:200,000) produced in these hearts very striking effects (fig. 1). Frequency was greatly augmented, and the contractions became uniform in size, if heart action was irregular before. Amplitude was frequently diminished, however, when barium was used. In some experiments the acceleration observed on perfusing the heart with barium chloride persisted after the perfusion was discontinued, but the diminished amplitude gradually returned to normal, or even greater than normal. That the diminution in amplitude was not an accidental occurrence was shown by the fact that the action was the same, when the perfusion with barium was repeated.

The turtle heart

The results obtained in experiments on the isolated heart of the turtle likewise indicated that the action of barium varied with the condition of the organ. When the fresh and untreated heart was perfused with barium in a concentration of 1:10,000 (or more dilute), no change could be observed if cardiac action was normal before the test was made (fig. 2). The effect was quite different, when the heart was slow and the contractions weak. Under these conditions perfusion with barium chloride was promptly followed by increased activity. Frequency was often greatly increased. One exception may be noted in which a heart that was not contracting for several minutes after removal

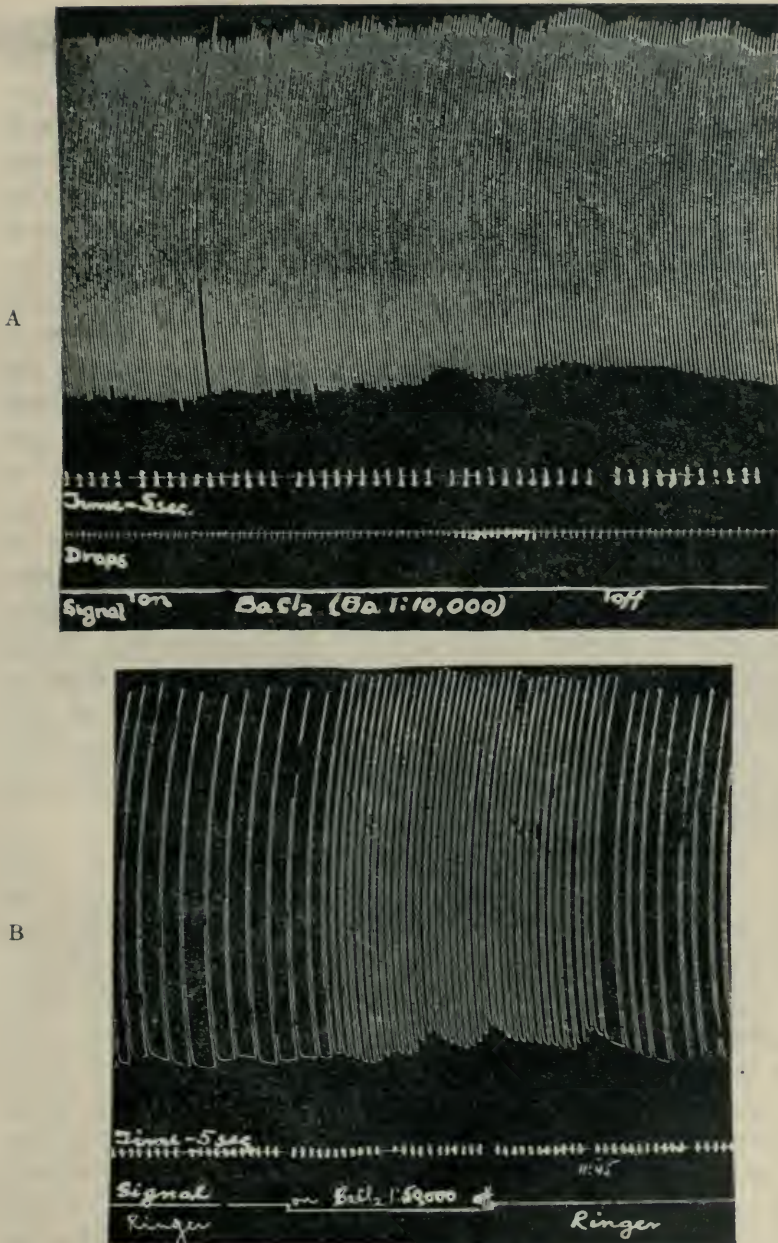


FIG. 2. FRESH UNTREATED TURTLE HEARTS PERFUSED WITH RINGER'S SOLUTION ALONE, THEN WITH RINGER'S SOLUTION CONTAINING BARIUM CHLORIDE

A shows that when heart action was normal barium chloride produced only a very slight increase in tonic. In B marked acceleration was produced by barium chloride, though concentration weaker than in A. Reduced one-half.

from the body failed to show any stimulating effect when perfused with barium chloride in Ringer's solution. In another experiment Ba in a concentration of 1:100,000 increased the frequency of the heart at first, but this was soon followed by retardation, reducing the number of beats about 30 to 40 per cent. The influence of barium on tonus was not constant. The same concentration of barium produced increased tonus in some experiments, while no effect could be observed in others.

THE ACTION OF BARIUM ON THE MERCURIALIZED HEART

To what extent the condition of the heart may influence the action of barium was shown in experiments in which its effects were tested after perfusion with mercuric chloride. As was pointed out by us in a recent report on the pharmacology of mercury (16) perfusion of the isolated heart of the frog and the turtle with some of the salts of this metal produced a variety of changes. Depression, diminished conductivity, and delirium cordis were observed after such treatment, the effect usually persisting after the perfusion with mercury was discontinued. Since the response to barium varied in the untreated heart with its functional state, it seemed desirable to study the effect of this metal on the abnormal heart. This was considered especially advisable in view of the fact that the heart often failed to resume its normal activity after treatment with mercury.

The frog heart

Perfusion with low and medium concentrations of barium chloride in Ringer's solution (usually weaker than Ba 1:5000) after treatment with mercuric chloride has invariably shown that the depression previously observed was greatly increased. Instead of the antagonism which might have been expected, there was an additive or synergistic effect. In one experiment in which the heart was perfused with mercuric chloride for nine minutes, the concentration of mercury being 1:50,000 in Ringer's solution, a steady decrease in the force of contractions occurred. When this was followed by barium chloride in Ringer's solution (Ba 1:200,000) the heart became still weaker, but it continued to beat

for several minutes. In another experiment paralysis occurred shortly after barium chloride was introduced into a heart previously perfused with mercuric chloride. In a third heart perfusion with Ringer's solution containing barium chloride (Ba 1:4000) and mercuric chloride (Hg 1:2000) caused paralysis almost instantaneously. It may be stated in this connection that a concentration of barium 1:5000 previously tried in the same experiment decreased the force of the heart, but not the frequency, and this was followed by recovery on resuming perfusion with Ringer's solution alone; while the concentration of mercury employed in this experiment, though strong enough to depress heart action, would be insufficient to cause paralysis almost immediately after its application.

The turtle heart

The reaction of the turtle heart to barium after perfusion with mercuric salts was found to be different from that obtained in the frog heart. The irregular action, slowing, and other manifestations of mercury poisoning were in a measure antagonized by barium chloride (fig. 3). The increase in frequency of the heart after barium became very pronounced in those specimens in which previous perfusion with mercury produced slowing or arrest of the heart, and in one case this occurred several minutes after the mercury treatment was discontinued, when the heart almost recovered. It may be observed, however, that in most cases the acceleration was associated with considerable diminution of amplitude. That the effect was due to barium and not to Ringer's solution was shown by the difference in the condition of the heart, when it was perfused with these solutions. If after perfusion with mercury Ringer's solution was passed through the heart for several minutes, the poisonous effects usually developed or became more pronounced. Perfusion with barium chloride was promptly followed by the changes described above. In two experiments only did we fail to obtain acceleration with barium chloride after previous treatment with the mercuric salts.

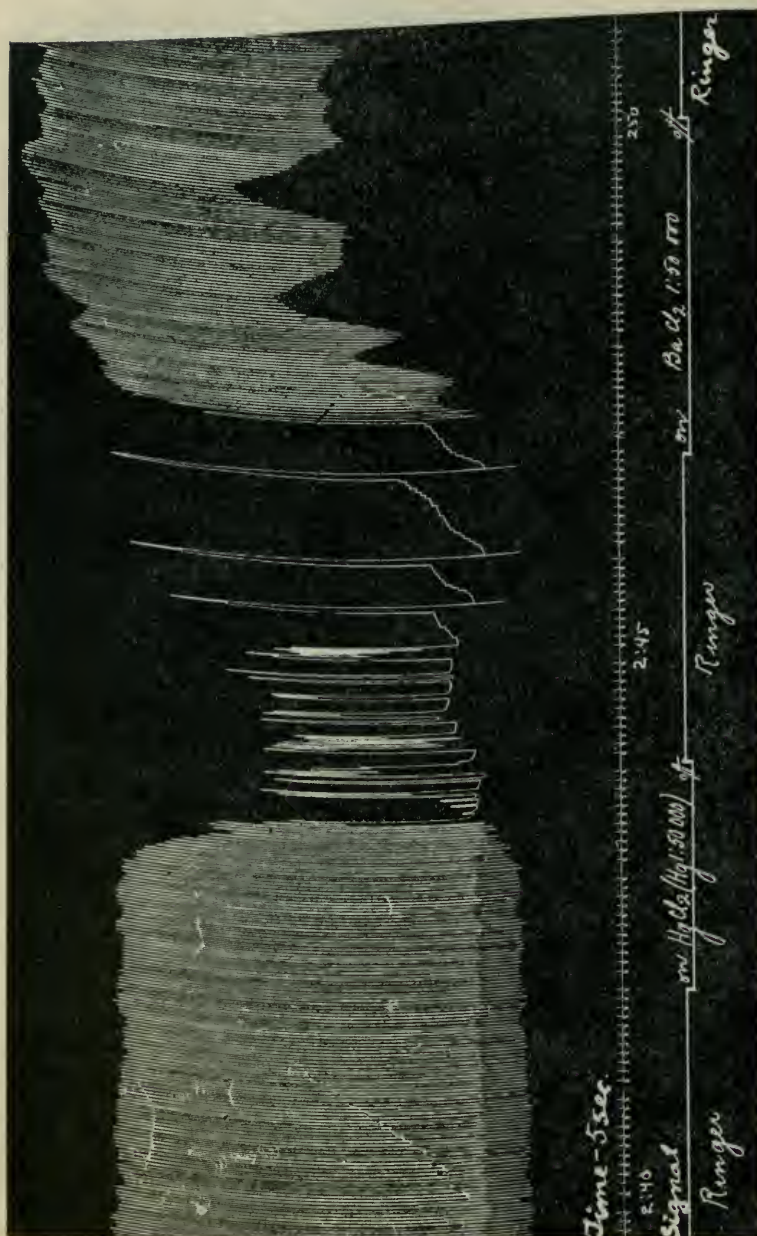


FIG. 3. TURTLE HEART

Delirium cordis, then heart block, when perfused with mercuric chloride was followed by Ringer's solution, but heart promptly restored by barium chloride, and tonus increased. Reduced one-half.

EXPERIMENTS WITH BARIUM AND ACONITINE

The results obtained with barium in the fresh and untreated heart have shown that acceleration occurred only when the rhythm was slow before the perfusion with barium chloride was begun. This was corroborated in experiments with mercury. In the frog heart, however, barium aided the depression in the force of the contractions caused by mercury without producing any change in the frequency, which may be accounted for by the almost normal rhythm observed before the test with barium was made. It was provisionally concluded, therefore, that barium probably exerts a moderating effect on the rhythm of the heart. This suggested the present series of experiments, since aconitine causes powerful excitation of the heart, especially augmenting its frequency.

The reactions to barium obtained in the frog and the turtle heart after it was perfused with aconitine furnished additional evidence that the pharmacological effect of drugs may depend upon the condition of the organ at the time it is exposed to the action of a particular compound. After the excitation and the delirium produced in the heart by aconitine barium caused changes which were wholly different from those observed in the untreated and in the mercurialized heart. Perfusion with barium after aconitine usually caused a very pronounced diminution of amplitude and also a considerable slowing of heart action. This was especially the case in the frog heart. The behavior of the turtle heart was less constant.

The frog heart

A brief description of only a few experiments is presented.

Experiment 132. Frog heart perfused with a saturated, but, of course, very dilute, solution of aconitine in Ringer's for four minutes. Heart action regular. Perfused with barium chloride in Ringer's solution (Ba 1:100,000) immediately after aconitine for three minutes. Slight increase in frequency, but amplitude diminished considerably. Recovery, when followed by Ringer's solution alone. Practically the same results obtained when the entire procedure was repeated.

Experiment 141. Frog heart perfused with aconitine in Ringer's solution for three minutes. Heart action very rapid, delirium, then slowing; amplitude increased. Perfused with barium chloride in Ringer's solution (Ba 1:50,000) for three minutes. Amplitude diminished even more than in preceding experiment, frequency moderately increased.

Experiment 171. Heart perfused with aconitine for seven minutes. Heart action very rapid, then delirium, followed by recovery. Perfused with barium chloride in Ringer's solution (Ba 1:50,000) for three minutes. Heart action irregular, and contractions weaker, but frequency showed little change from normal. Recovery with Ringer's solution alone.

The turtle heart

As the results obtained in experiments on the turtle heart were in some respects different from those observed in the frog heart when barium was used after aconitine a more detailed description is given. It may be pointed out in this connection that the effect of aconitine on the turtle heart has, as far as we know, never been reported before.

Experiment 182. Repeated perfusion of the turtle heart with aconitine and barium chloride in Ringer's solution. Perfusion of the untreated heart with barium chloride (Ba 1:10,000) caused increased tonus and decreased amplitude of the contractions. Aconitine tried later stimulated tonus, but no other effects were observed. Several minutes later, perfusion with the same concentration of barium chloride as before produced cardiac irregularity. Heart action became rapid at first, but the contractions were much weaker. This was soon followed by increased force and diminished frequency of the contractions. A second trial with aconitine, then with barium produced essentially same effects. In this case the heart showed considerable resistance to aconitine, but when this was followed by perfusion with barium, cardiac irregularity developed.

Experiment 183. Perfusion of the turtle heart with aconitine was not followed by any immediate changes. When perfused later with barium chloride (Ba 1:50,000) heart block and delirium cordis developed.

Experiment 135. Turtle heart perfused with barium chloride (Ba 1:100,000) for four minutes. Tonus waves became very pronounced, the contractions less frequent and weaker than before. After perfusion

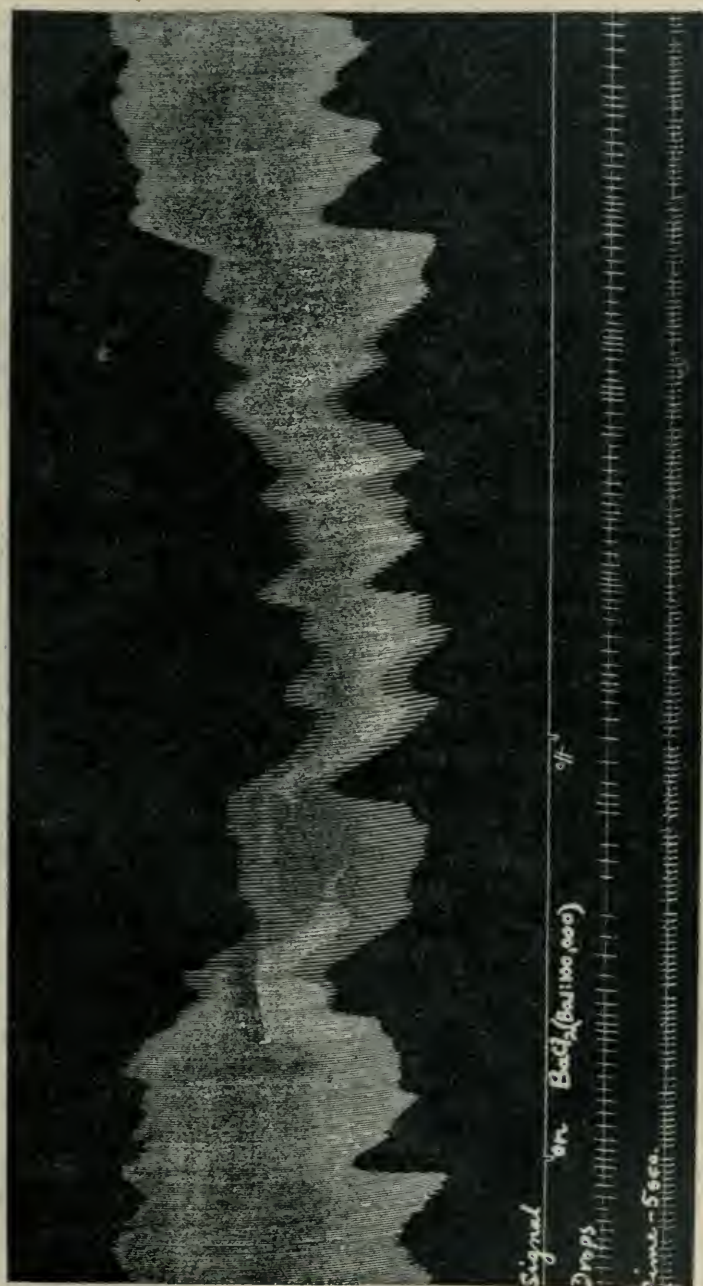


FIG. 4. TURTLE HEART PREVIOUSLY PERFUSED WITH ACONTINE (NOT SHOWN IN FIGURE)

Heart block, but no delirium. Perfusion with barium chloride caused depression. When barium was replaced by Ringer's solution alone, the heart gradually recovered. Reduced one-half.

with aconitine later heart action became extremely rapid. When followed by Ringer's solution irregularity also developed. Perfusion with the same concentration of barium chloride as before abolished the irregular action of the heart and also increased the frequency. This test with aconitine and barium was repeated on the same heart. The very rapid heart action developed by perfusion with aconitine was again slowed under the influence of barium chloride.

In three other experiments barium chloride following perfusion with aconitine produced cardiac depression and irregularity. In two specimens the contractions became weaker, but there was no change in frequency. In a third marked slowing of the rhythm developed after barium chloride (Ba 1:50,000) was perfused for two minutes. Group contractions also occurred.

EXPERIMENTS WITH BARIUM AND COCAINE

The influence of cocaine on barium was studied in three turtle hearts and in one frog heart. The slowing of the heart and the partial heart block which was caused by perfusion with cocaine for a very brief period¹ lasted in some instances as long as fifteen minutes. Nevertheless perfusion with barium chloride after cocaine was without any effect. Occasionally, however, some elevation of tonus was observed when the heart was perfused with barium chloride some time after cocaine, when the effects of the latter almost disappeared. If barium and cocaine were used together, some potentiation occurred. Slowing of the heart became more pronounced, while its action was also more irregular.

Experiment 195. Perfusion of the untreated heart, action normal, Ba 1:10,000, had no effect. A few minutes later the action of cocaine 1:1000 was tested. The heart was perfused for two minutes. No change was observed for nearly two minutes, but at this time the rhythm suddenly became slower, the amplitude increased, and incomplete heart block developed. This condition persisted with hardly any change for about fifteen minutes, when the frequency was slightly increased and the contractions became somewhat weaker. Two minutes later perfusion with barium chloride (Ba 1:10,000) for three minutes

¹ Unpublished results, to be reported soon.

produced a still greater decrease in amplitude and increase in tonus, but the frequency of the heart remained the same. Perfusion with barium chloride was repeated twenty minutes later. The effect was the same, though the rate of the heart was more rapid before the perfusion with barium. A considerable change in heart action occurred, however, when barium and cocaine were perfused together. After a latent period of nearly two minutes a very pronounced slowing of the heart took place. The number of contractions was rapidly reduced to four per minute. The retardation persisted for a considerable length of time after the perfusion, as in the first test with cocaine. Heart block also developed. That this action was due to the combined effect of barium and cocaine was shown later, when the heart was again perfused several times with cocaine alone. There was a very pronounced slowing in each case, but this was less than after perfusing with both drugs.

EXPERIMENTS WITH BARIUM AND ACONITINE ON THE ISOLATED INTESTINE

The tests were made on the small intestine of cats and rabbits which were killed by decapitation. Segments about 4 cm. long were then removed and suspended in a Harvard muscle warmer containing Locke's solution through which a constant stream of air was passing. The temperature of the solution was maintained at 37 to 39°C. for the duration of the experiment. The contractions of the intestine were recorded in the usual fashion. Since the behavior of the intestine of the cat and of the rabbit showed well marked differences in these experiments, a separate description of their reaction to the drugs will be given.

Intestine of the cat

Segments of the small intestine of the cat suspended in Locke's solution either failed to contract for several minutes, or their movements were slow and feeble. Upon the addition of a saturated solution of aconitine a distinct improvement occurred. After five or ten drops of this solution were added to 30 cc. of Locke's, the intestine began to contract, when there were no movements before, or a noticeable increase in the force and frequency of the contractions took place, when these contractions

were slow and weak at first. It may be added that tonus was, on the contrary, depressed, the intestine remaining in this condition for several minutes, while executing much more forcible contractions than before aconitine. The depression of tonus, however, was not constant. The stimulating effect of aconitine continued for a period of several minutes, when the movements began to show signs of weakening. But upon the introduction of more aconitine the intestinal movements were again stimulated. After a considerable amount of aconitine has thus been added to Locke's solution stimulation gave way to depression, and the contractions of the intestine became weaker and less frequent. The action of barium chloride on intestine previously subjected to the action of aconitine was studied under these conditions. Small amounts only (1 mgm. barium in the form of the chloride) were introduced into the cylinder containing 30 cc. Locke's solution. Even these quantities of barium produced a very marked reaction. The intestine promptly contracted upon adding the salt, but this was immediately followed by relaxation (fig. 5). The rhythmic contraction observed before barium was introduced were either entirely abolished, or became still weaker, but more frequent.

Intestine of the rabbit

The effects of aconitine on the isolated intestine of the rabbit were as follows: Small quantities (5 to 10 drops of the saturated solution) produced a very pronounced relaxation which was sometimes preceded by initial tonic contraction of very short duration. The rhythmic movements showed a considerable change in character. They became less irregular and sometimes more frequent than before aconitine. Large tonus waves observed before aconitine completely disappeared after a total of 3 cc. of the saturated solution of the alkaloid was introduced into the cylinder containing 30 cc. of Locke's solution. The contractions became much smaller, being almost uniform in size and of regular rhythm. When 1 mgm. barium was now introduced, tonus was stimulated for a brief period, and this was succeeded by a gradual depression with complete disappearance of the rhythmic contrac-

tions. In another experiment the same treatment produced different results. Barium after aconitine stimulated tonus and restored the rhythmic contractions which were previously abolished as a result of repeated treatment with aconitine. It

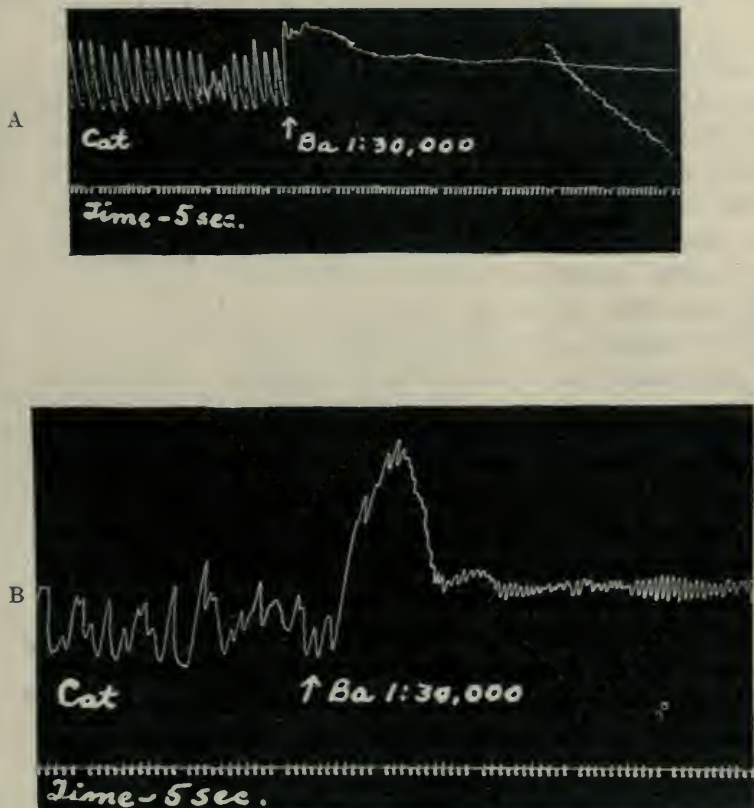


FIG. 5. SEGMENTS OF SMALL INTESTINE OF CATS IN AERATED LOCKE'S SOLUTION

Barium chloride added after aconitine caused complete abolition of contractions (A). In B barium after aconitine produced acceleration, but amplitude very much decreased.

may be added, however, that this was the only case in which rhythmic contractions occurred in the intestine after barium chloride, when none were observed before, in the presence of aconitine.

DISCUSSION AND CONCLUSIONS

The evidence presented in the foregoing experiments warrants the conclusion that the action of barium varies with the condition of the organs with which it comes in contact. This was suggested by the results obtained in experiments on the fresh and untreated heart, for if barium was used when heart action was slow or altogether absent stimulation was readily obtained; on the other hand, no change could be observed when heart action was normal before perfusion with barium chloride. That the effect of barium depends upon the state of the tissues to which it is applied was further shown when it was administered after mercury or aconitine. On perusal of the experiments cited the reader will see that barium caused stimulation after mercury, the frequency being increased, as in the slowly contracting fresh heart, when similarly treated. But this occurred in the turtle heart only. In the frog heart, in which mercury produced depression of the amplitude without causing any significant change in rate, barium caused further decrease in the strength of the contractions, the frequency of the heart remaining without much change until paralysis set in. Quite different was the action of barium after previous treatment with aconitine. Heart action which was rapid and irregular (especially in the frog heart) became slower and regular, and the amplitude was considerably decreased. Attention may be called to the difference in the behavior of the frog and the turtle heart in their reaction to aconitine under these conditions. The turtle heart often exhibited considerably greater resistance to the action of aconitine than the frog heart. It is of interest to observe that the action of barium also differed in the hearts of these animals, the depression produced being less constant in the turtle than in the frog heart.

That the depression produced by barium is not confined to the heart, when previously treated with aconitine, was shown by experiments on the isolated intestine, particularly the small intestine of the cat in which barium produced marked depression after aconitine. As is well known and as was shown by Salant and Mitchell (17) barium causes stimulation of the normal isolated intestine.

Cocaine scarcely produced any change in the action of barium on the heart. Some synergistic action, however, was obtained when the two drugs were administered together.

The data presented justify, therefore, the conclusion that barium may cause stimulation as well as depression. The results obtained by Poulsson (3), Werschinin (4), and Trendelenburg (5) are of interest in this connection. They pointed out that the action of barium varied according to its endocardiac or exocardiac application, causing a diastolic effect in the first and a systolic effect in the second case. It was also shown that these effects were due to the difference in the permeability of the endocardium and exocardium.

Is it not possible that a similar mechanism is operative when different organs are subjected to the action of barium after aconitine or mercury, or even when barium is used on the untreated heart in various conditions of functional activity? The numerous observations on the permeability of the cell made by different workers would, indeed, seem to indicate that chemical substances as well as physical agents change the permeability of the cell. Perhaps similar factors were present in our experiments, resulting in changes of permeability to different ions, modifying the action of barium and thus causing stimulation or depression.

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THE COMPARATIVE CONCENTRATIONS OF ALCOHOL IN HUMAN BLOOD AND URINE AT INTERVALS AFTER INGESTION

WALTER R. MILES

*Nutrition Laboratory of the Carnegie Institution of Washington, Boston,
Massachusetts*

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It is well known that ethyl alcohol introduced into the stomach begins quickly to be absorbed, to appear unchanged in the blood, to be utilized in the metabolism, and, if given in sufficient amount, it quickly produces characteristic subjective effects. Dogiel (15) reports finding alcohol in both arterial and venous blood within two minutes after ingestion and Higgins (34) at the Nutrition Laboratory showed that it "begins to be burned in appreciable quantity in from five to eleven minutes after taking." Utilization, at first, does not keep pace with absorption and the content in the blood gradually rises to a maximum near which it may remain for a period of one or more hours. This stage is designated "the Gréhant plateau" (22), (27), (80). After this there is a slower decrease in alcohol content, the base line, or complete disappearance of the alcohol from the blood being reached only after several hours (21). The time relations of this cycle and the height of alcohol concentration attained depend upon the amount, in terms of body weight (23), and the concentration (44) of the alcohol ingested, the food and liquid previously taken (72) and several other factors (58).

Not only is the alcohol found in the blood but it is recoverable from the other body fluids and tissues, particularly at or following the time when it appears maximally in the blood. Fridman (18) and Vollmering (69) among others have studied its content in the tissues and emphasize the relatively large amount found in the brain. Nicloux (49) demonstrated its presence in

the lymph, saliva, pancreatic juice, bile, cerebro-spinal fluid, amniotic fluid, and milk (48) as well as in the urine, where it had long been known to appear. These experiments were on dogs and guinea pigs. From 3 to 5 cc. of absolute alcohol were given per kilogram of body weight and the comparison samples, blood and other fluid, were taken one to three and three-quarter hours after ingestion, i.e., within the "periode du plateau." Exclusive of milk, the body fluids show an average alcohol content of 3.47 mgm. per cubic centimeter of fluid and the blood gave an average of 3.42 mgm. per cubic centimeter identity which is very striking.¹ Nicloux concluded that blood and urine had about the same alcohol content, and several years later when with Nowicka (56) he made a study of the permeability and absorbing power of the bladder for alcohol, he found no reason to change the statement. Although in certain of their experiments the alcohol in the urine exceeded that in the blood by about 25 per cent, Nicloux and Nowicka held that "the urine secreted by the kidney has probably the same alcohol content as the blood, unless we admit a selective power of renal epithelium for alcohol which remains to be demonstrated." Widmark took up this problem and in two papers (76), (78) summarized existing data and contributed some results on human subjects. He concluded the concentration of alcohol in urine excreted in short intervals agrees very nearly or completely with that of the blood and that a knowledge of the alcohol content of the organism after the taking of alcohol can therefore be obtained from urine samples. Chabanier and Ibarra-Loring (10) claim to have independently substantiated this result and it has been accepted in recent studies as for example those of Seppä (64) and Haneborg (29) while Ambard (2) has placed much emphasis upon it, holding that ethyl alcohol belongs to a class of substances which the kidney eliminates wholly by *diffusion*, i.e., without any *concentration* of the substance in the urine.

¹ For the sake of direct comparison all the results or data quoted in this article are given in terms of alcohol by weight. Since the specific gravity of alcohol = 0.793, it is usual to consider that the percentage by weight = percentage by volume $\times 0.8$.

Gréhant (27) and particularly Schweisheimer (63) contend that the curve for alcohol concentration in the blood is parallel throughout its course with that for intensity of intoxication or alcohol effect. Schweisheimer says that this fact stated by him for the first time, could not be proven with animal experiments, but that his work with men, abstinent, moderate drinkers, and habituals, absolutely proves that the intoxication is "exactly parallel in increase and decrease with the rising and sinking of the alcohol content in the blood." Such a relationship may seem a logical one judged by clinical experience but so far as the writer is informed no strictly objective data has ever been published which establishes this correlation. Schweisheimer's subjects were kept recumbent in bed during the experiments for the obvious purpose of reducing and making uniform the factor of muscular exercise. By this routine the opportunity of observing and estimating the degree of intoxication must have been rather limited, and his positive statements seem unwarranted. In the recent work of Mellanby (45) it was found that the incoördination (alcohol effects) began to decrease and disappear immediately after the alcohol in the blood had reached a maximum, i.e., before it began to decrease in the blood.

The problem of relating the intensity of physiological or psychological effects to the incidence and concentration of a substance like ethyl alcohol in the blood is relatively new and in this case unsettled. It merits much care being expended on the determination of the alcohol, in relation to the time, the concentration of dose, and conditions of ingestion. And since urine samples are so easily available in such an experiment it is important to know exactly how the urine content stands in relation to that of the blood so that, if possible, the urine samples may be sufficient for making the comparison with the intensity of neuromuscular disturbance. A man who is being tested for the effect of alcohol on his efficiency is certain to have his performance disturbed, in many cases considerably modified, if frequent blood samples must be taken. Perfect identity or elimination by diffusion therefore becomes a question of importance to one who is interested in such experiments. As most substances excreted

in the urine are present in from two to sixty times the concentration found in the plasma, Cushny (11), it seems clear that alcohol is rather exceptional and in strong contrast to such a substance as urea in this regard. It was probably more or less from this standpoint that Nicloux and Widmark have viewed the matter. Ambard, however, holds for perfect and complete diffusion stating that the kidney is in this instance like an inert membrane, and that the process is purely physical, very rapid, and a definite abrupt departure from excretion by secretion.

In no published experiments have more than three blood samples been taken for comparison with the alcohol content of urine samples. There are but two or three such comparisons available, mostly only a single blood sample was secured one and one-half to eight hours following ingestion. To the writer the evidence appears fragmentary and inconclusive, particularly in reference to the relative content and time relations during the first hour after alcohol ingestion. The present paper is devoted to this problem of comparison of blood and urine; the relationship of any curve for alcohol effect with the alcohol content in the blood will have to be reserved for later presentation.

METHOD AND PROCEDURE²

1. *The Widmark-Nicloux method of alcohol determination*

The reaction of alcohol with potassium bichromate solution in the presence of sulphuric acid is the basis of a method employed in a large number of the papers contributed during the last forty years to the study of the physiological presence, elimination, distribution, and fate of alcohol.

Nicloux (49) arranged a technique for carrying out the reaction simply and in such a way as to provide a *direct* volumetric

² For my first introduction to this method, I am indebted to my colleague, Dr. T. M. Carpenter, and his two former assistants, Miss J. L. Finn and Mr. W. M. Konikov. I desire also to record the pleasure and profit received from a personal visit with Professor Widmark at his laboratory in Lund in July, 1920, when he showed me every courtesy. Many of the analyses presented later have been made by or with the help of my assistant, Miss E. L. Fruitkoff, whose accurate work I gratefully acknowledge.

determination of the alcohol in the sample. The bichromate is added, a drop or two at a time, heating gently to boiling, and shaking between additions, until the blue-green just changes to yellow-green due to the slight excess of the bichromate. This change in color, which Nicloux claims he was the first to use in this way (51), is an end point which is quite sensitive. In later papers (52) (55) Nicloux has recommended the use of more dilute bichromate solutions (3.8 grams $K_2Cr_2O_7$ per liter) which daapt the method for working with small quantities (1 cc. or less) of distillate containing only traces of alcohol. But in all cases the intensity of the blue-green or yellow-green color is dependent on the amount of bichromate which is necessary to be added to a given sample. Thus the saturation of the color is not constant from sample to sample and in titrating, one watches for a *color change* which is rather slight, and has offered difficulty to some workers.

Bodländer (9), Cotte (58), Benedict and Norris (5), Pringsheim (58), and Mellanby among others have introduced the distillate into a *certain* amount of potassium bichromate and concentrated sulphuric acid. Bodländer compared the green color obtained from a sample with a series of previously made standards. The others estimated the excess of bichromate by titration against ferrous ammonium sulphate, Cotte, Pringsheim, and Mellanby "spotting" against ferricyanide during the latter part of the titration and obtaining a sharp end point with the formation of prussion blue. Benedict and Norris add an excess of ferrous ammonium sulphate and determine this by titration against a potassium permanganate solution, the end point being a sudden change from faint blue or green to deep purple.

Widmark (79) in 1916 modified the "Nicloux method" so that the end point is found by matching with a standard color. He follows Bodländer and the others in so far as using a certain amount of bichromate and sulphuric acid into which the alcohol from 5 cc. of the sample is distilled; but the excess bichromate he reduces by adding known alcohol until a standard color is reached. The alcohol added from the burette is subtracted from the weighed amount known to be necessary to reduce the

bichromate used. The remainder, therefore, is the milligrams of alcohol contributed by the sample.

As employed in my experiments, Widmark's technique was modified, chiefly in four respects:

(a) To each sample when prepared for distillation 5 cc. of concentrated solution of picric acid were added. The picric acid caused the precipitation of the proteins in the blood and made possible a colorless distillate. It had the further advantage of reducing frothing more successfully than talc or kaolin, and that the blood did not become so dry during heating. The picric acid was added to urine, plasma, whole blood, and also when making up the standard for the day. *Thus the routine was identical with all kinds of samples.*³

(b) The heating was more mild and prolonged. The sample to be distilled always had a volume of 10 cc. (5 cc. of sample + 5 cc. of picric acid) and was placed in a 100-cc. round bottom flask. The flame was regulated from time to time so that the rattling noise would start about two and a half minutes after heating had commenced. A second stop-watch was started, when rattling began and the heating continued two and a half minutes longer. By the five minutes of heating about 2.5 to 3 cc. of liquid ($\frac{1}{4}$ of the sample) were distilled over into the test-tube which already contained 5 cc. of H_2SO_4 and 1 cc. of $\text{K}_2\text{Cr}_2\text{O}_7$. Nicloux (55) has claimed that to distill over $\frac{1}{7}$ to $\frac{1}{8}$ of the sample takes all the alcohol.

(c) More dilute potassium bichromate was used—1 cc. of 1.15 per cent—than Widmark or Nicloux suggest and the standard was made by adding 2.5 mgm. of alcohol. The practical alcohol limit in the urine and other samples without dilution was therefore a little less than 0.5 mgm. per cc. As our samples usually showed an alcohol content below this, the dilute bichromate gave greater accuracy to the determinations.

(d) All samples being titrated were kept with the standard in a hot water bath, therefore, changes in color due to cooling as

³ This procedure was arrived at independently at the Nutrition Laboratory, but Nicloux (53) (55) recommended the addition of picric acid, 13 to 15 cc., to all samples of blood, urine, and tissue before distillation.

Widmark found did not take place. Several daily standards kept over a period of four weeks when warmed were found practically identical in color between themselves and with a fresh standard. Six burettes graduated to 0.02 cc. were filled with the 5 mgm. per cubic centimeter alcohol solution and as many samples could be in course of titration at once. After each addition of alcohol care was used that *all the alcohol introduced* including any on the side of the test-tube should be thoroughly mixed by shaking with the chromic acid and the test-tube was placed for some time in the hot bath before being compared with the standard. The colors were usually matched by good daylight but comparisons made by artificial light showed no necessity of adhering to such a condition.

The plasma distillations gave some difficulty, otherwise, the method worked very satisfactorily and the procedure could be carried out quite rapidly. The speed with which such analyses may be made is a consideration since it is inadvisable to add any preservative to the samples. These were kept on ice and the alcohol determined within twenty-four to thirty-six hours after collection. One experiment required from thirty to forty determinations.

2. Accuracy with which control samples of known alcohol content could be analyzed

The accuracy of the method is, of course, of prime importance and also it must be workable with small total amounts of alcohol. Controls were rather frequently introduced along with the urine and blood samples. The weights of alcohol actually added to the water, urine, or blood varied from 0.10 to 0.45 mgm. per cubic centimeter and were unknown to the one making the analysis. I have gathered the results for all the determinations of these "knowns." There are 86 such cases and as no such trial has been discarded, this series will show what can be expected from the method. In figure 1, the 86 trials are classified separately accordingly as the alcohol; was diluted with water, urine, or blood, and are arranged in the form of three distribution areas from which one can immediately see the approximate range and

amount of error. The figure is divided by a vertical line separating minus errors from plus errors. The squares marked off in the column designated 2 per cent minus represent those cases in which the analysis showed 2 per cent or less below the actual

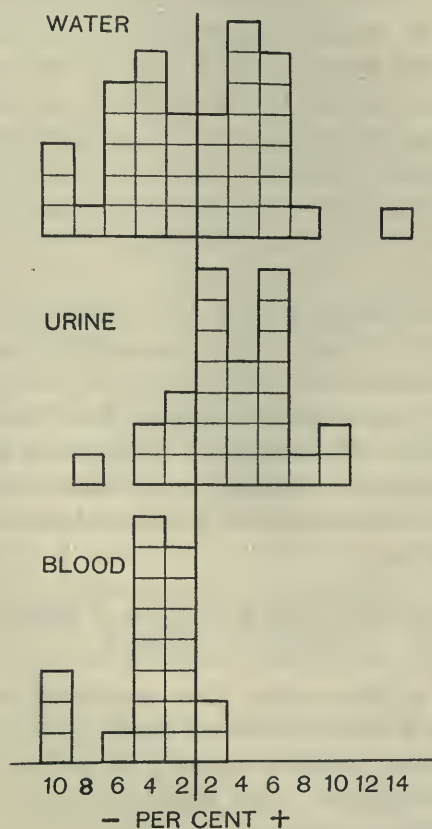


FIG. 1. ERRORS IN THE ANALYSIS OF 86 CONTROL SAMPLES MADE UP WITH KNOWN ALCOHOL CONTENT

A demonstration of the accuracy of the Widmark-Nieloux method of alcohol determination.

amount of alcohol added. There were a few cases in which the determinations came out exactly correct. These have been equally divided between the 2 per cent minus and the 2 per cent plus columns. There were 38 cases made up with water; 18

showed minus results, averaging 4.4 per cent; 18 showed plus results, averaging 4.1 per cent, and 2 zero errors. In 27 controls made up with normal urine, 6 were minus with an average of 2.7 per cent; 21 were plus with an average of 3.7 per cent. There were 21 cases of "known" alcohol added to normal blood samples; 18 showed minus results, averaging 3.5 per cent; only 1 showed a plus result which was 1.2 per cent and there were 2 cases with zero errors. If the 86 cases are taken as one series the average error is ± 3.6 per cent. The original Nicloux method was said by its author (49) to give results to within 2.5–10 per cent; tending towards the 2.5 per cent or below this after much practice.⁴ Fridman (18) and others using it claimed accuracy to within 5 per cent. Widmark (79) publishes results for 60 "knowns" where from 0.25 to 1.50 mgm. per cubic centimeter of alcohol had been supposedly added to water. The whole series as printed shows an average error of ± 5.4 per cent. Considering the 20 cases where the samples contained 0.25 or 0.50 mgm. of alcohol per cubic centimeter and with his bichromate solution I (1 cc. of 2.3 per cent) the average error was ± 4.3 per cent. There was a definite tendency for these results to be minus on the average — 3.4 per cent. It is common experience with all distillation methods to recover less alcohol than is put into the sample, this shows up very slightly, about — 0.2 per cent, in my own series of 38 water-alcohol controls. Widmark accounts for his rather large minus error on the supposition that the standard gradually becomes more blue, and that loss of alcohol takes place from the titration liquid.⁵ But since he titrated immediately after distillation and without further heating it is almost certain that too much alcohol will be admitted if the sample is

⁴ Hanzlik (30) has described a modification of the bichromate-sulphuric acid test for alcohol with which he claims an accuracy of 1 per cent.

⁵ If a rather long test-tube of small diameter is inverted and slipped over the top of a burette, it is practical then to leave the burette filled to the very top and at the commencement of making a series of determinations to draw off enough to bring the meniscus to a convenient position. The burettes thus keep clean and ready for use, but the titration solution must be checked frequently as the alcohol gradually decreases, Nuss (57), in these very dilute solutions, and especially so if allowed to stand in a burette for several weeks without use.

brought quickly to the standard color. By slower titration and the use of a hot-water bath this defect in the method is very much reduced.

The normal presence of alcohol in the body tissues and fluids has been much discussed since the work of Ford (16), (17). Atwater and Benedict (3), Landsberg (38), Beach (59), and many others (Schweisheimer reviews this topic) have found substances present which act like alcohol in that they reduce potassium bichromate. Our experience has been the same. From a group of 26 normal urine samples which represent 2 or more from each of 8 men we found as an average, reducing substance equivalent to 0.017 mgm. per cubic centimeter. In presenting our results on controls and in the experiments later recorded no deduction has been made for this factor of normal reducing substance. On a basis of 0.40 to 0.50 mgm. the value 0.017 would account for the positive error in the analysis of known urine samples as against the alcohol-water samples.

While the amount of reducing substance in the blood is less than that in the urine the difference is not sufficient to account for the discrepancy found between blood and urine and we are at present unable to fully account for this. It is recognized that alcohol can be oxidized in the liver, Hirsch (36), after this organ has been removed from the body, but it seems improbable that such a process should go on in the red cells and particularly after potassium oxalate has been added. Martenstein (43) was able to recover alcohol about equally well from solutions with olive oil or water but there was a larger percentage of loss in recovering it from blood. In the following experiments, we regularly redistilled all the blood samples, adding 5 cc. of water after the first distillation and distilled into a fresh tube of bichromate, to satisfy ourselves that the alcohol had been really recovered by the first distillation. These second distillations were titrated and without exception gave only blanks.

3. The human subjects used

The men used in this investigation were mostly medical students who had previously given blood samples for transfusion or

other purposes. They were thoroughly coöperative, reliable subjects and their statements are trustworthy. All were in good health and ranged in age from twenty-two to thirty-six years with the exception of *Kac* who was fifty-two years old and of the habitual class. Each man was seen individually some days before coming as a subject and all details were gone over and agreed upon. Practically all had taken alcohol as a beverage at some time in their lives or were in the habit of taking it occasionally and nearly all had experienced mild or average intoxication from alcohol.

While groups of people who are of abstinent or very moderate habit can be readily secured, it is difficult to obtain subjects of the so-called habitual class who will coöperate, whose statements can be trusted, who are willing to abstain from alcohol during the forty-eight hours prior to an experiment and to endure the discomfiture of blood samples. Added to this, one wishes that the subjects shall be in fairly normal health, not hospital cases. To represent this class we have two subjects only, a man who has been in the habit of frequently taking large quantities of alcohol (he shows some tremor) but whose coöperation could be counted on, and a younger man, a very intelligent student, who is known to be a heavy occasional drinker.

4. General experimental routine

A man came to the laboratory having taken food three hours or so previously and having refrained by agreement from alcohol, coffee, and tobacco. He emptied the bladder, and his time was then employed for about one hour in the performance of some neuro-muscular tests. These did not require strong physical activity as all were made with the subject sitting at a desk or at an instrument. There was a slight amount of movement from one table to another and of walking back and forth to the adjoining room where the urine samples were collected. Aside from giving the subject practice in the tests to be used in later experiments, they here provided a standard amount of activity and served to distract somewhat his attention from the taking of the blood and urine samples.

After one hour of testing, the bladder was emptied, this urine being saved as a normal. Urination was always directly into glass cylinders, graduated in cubic centimeters and provided with glass stoppers. Immediately after passing this urine, the subject drank the dose already prepared for him. At just twenty minutes after the dose had been completely ingested, a blood sample of 25 to 30 cc. was taken from an arm vein in the usual manner. Just enough potassium oxalate was introduced into the syringe to avoid coagulation of the blood. The arm had, of course, been previously washed and thoroughly dried. The blood sample actually was drawn during the period of about one-half minute and immediately following this, the subject gave attention to passing a urine sample. This was usually possible from one to two minutes after the needle had been withdrawn from the vein.

Blood samples were as a rule taken 20, 40, 70, and 120 minutes, and urine samples were passed 22, 42, 55, 72, 87, 105, 122, 140, and 160 minutes after finishing the dose of alcohol. Each time the bladder was as completely emptied as possible. Usually the amounts of urine were quite sufficiently large for the purpose of analyses. During all of the intervals when blood or urine samples were not requiring the attention of the subject, his time was taken with repeating the various neuro-muscular tests.

5. Weight of ethyl alcohol employed

A uniform amount of alcohol which equalled approximately 0.5 cc. per kilogram of body weight (1) was used with each subject. Always the same dose was given to all the subjects on the first day. This was one liter of 2.75 per cent alcohol by weight, i.e., 27.5 grams of absolute ethyl alcohol were added to 300 cc. of grape juice and this solution of grape juice and alcohol diluted with tap water to a total of 1000 cc. The alcohol content of the grape juice was controlled from time to time. An average of several such analysis gave 0.087 per cent alcohol by weight, in all cases tried less than 0.10 per cent. The grape juice had solids of about 18 per cent and was not sweetened. It was desirable to use some such fruit juice with the alcohol since plain alcohol

and water mixtures are very unpleasant to take. The liquid was consumed at 18 to 20°C. The subject was not rushed in drinking. In all cases three to seven minutes were sufficient time for ingesting the liter of fluid. Following ingestion there was some feeling of cold. This volume was not found to be excessive for any subject. In the Volhard (68) test it will be recalled that 1½ liters of water are given, and Vernon (67) found it possible to ingest 2 and sometimes 3 liters of 3 per cent beer or cider.

The second experimental day did not always immediately follow the first, but on it a concentrated dose of 100 cc. of 27.5 per cent alcohol by weight was always given. This was made by adding 27.5 grams absolute ethyl alcohol to 50 cc. of grape juice and diluting this to a total volume of 100 cc. with water. It was taken at from 17 to 19°C. at one time and, of course, easily within less than one minute. No additional water was taken following this although in one case the subject was allowed to rinse out the mouth. The concentrated dose was not relished by the more abstinent men; on the contrary, the habituais preferred it to the more dilute beverage.

THE EXPERIMENTAL DATA

1. Preliminary comparison on a moderate drinker thoroughly practiced in the experiments

The first occasion on which blood samples were taken for a direct comparison with the alcohol content of urine samples was on April 15, 1921. It was done with the idea of confirming previous results and of having some original data to which one could point when stating the conclusion of Widmark (81) and Ambard (2) that the alcohol content of simultaneous urine and blood samples is identical.

Experiment 1. The subject, *Mow*, is a moderate user of dilute alcoholic beverages. Age thirty-six years, height 178 cm.,⁶ weight 74 kgm., excellent health. He had a hearty breakfast at 7:30 and a

⁶ The heights are always without shoes. The weights are without shoes, coat, or vest, after emptying the bladder and before drinking.

light lunch consisting of four small crackers and a little jam at 11 o'clock. At 12 o'clock noon, a normal blood sample was taken. At 1:00 the bladder was emptied, urine was saved, then the regular 1-liter dose, i.e., 27.5 grams absolute alcohol and 300 cc. of grape juice diluted with water to 1000 cc., was taken and finished at 1:10. The time of taking the samples, the period covered by each, the volumes of urine, the amount of urine passed per minute, the titration figures, and

TABLE 1

A typical table giving the protocols, titration figures, and final analytical results of one experiment in which the alcohol concentration of blood and urine is compared

SAMPLE	TIME		VOLUME URINE		TITRATION		ALCOHOL PER CUBIC CENTI- METER
	End	Duration	Total	Per minute	1	2	
		<i>minutes</i>	<i>cc.</i>	<i>cc.</i>			<i>mgm.</i>
Blood.....	12:00				0.49	0.48	0.005
Urine.....	1:00		45		0.49	0.49	0.010
Dose.....	1:10						
Urine.....	1:30	30	11	0.4	0.31		0.238
Urine.....	1:45	15	9	0.6	0.31	0.32	0.224
Urine.....	2:00	15	10	0.7	0.22	0.28	0.275
Urine.....	2:15	15	26	1.8	0.10	0.10	0.400
Blood.....	2:31				0.29	0.30	0.202
Urine.....	2:32	17	82	4.8	0.07	0.08	0.424
Urine.....	2:47	15	59	3.9	0.09	0.08	0.413
Blood.....	3:03				0.34	0.33	0.158
Urine.....	3:04	17	9	0.5	0.20		0.375
Urine.....	3:38	34	51	1.5	0.13	0.12	0.373
Blood.....	4:01				0.36	0.38	0.123
Urine.....	4:02	24	44	1.8	0.17	0.16	0.332
Urine.....	4:32	30	41	1.4	0.22	0.23	0.275
Urine.....	5:05	33	22	0.7	0.37	0.36	0.128

the alcohol content expressed in mgm. per cubic centimeter for blood and urine are given in table 1. Blood samples of 25 to 30 cc. were taken at 2:31, 3:03, and 4:01 p.m. The analyses were made in duplicate as was our custom and the titration figures show very close agreement. In the case of the 2 o'clock urine, different amounts were used in the two analyses, hence the titration figures do not agree as they stand in the table. However, the two determinations gave 0.276 and 0.275, the average of which is stated as 0.275.

On alternate periods of this day, the subject did a definite quantity of work on a bicycle ergometer and the work period alternated with the psychological tests. The three blood samples taken after the alcohol was ingested were in each case drawn immediately after a work period while the subject was still sitting on the bicycle and were followed as nearly as possible by urine samples as table 1 reveals. The comparative results may be most easily seen in graphic form, figure 2. The left hand scale is for mgm. of absolute alcohol per cubic centimeter of blood and urine. The right hand scale in cubic centimeters per minute is for urine per minute, which curve is drawn in blocks. The abscissa shows minutes after ingestion of the alcohol. The normal blood and urine are practically perfect blanks. The curve showing the concentration of alcohol in the urine is plotted on the points when the urine sam-

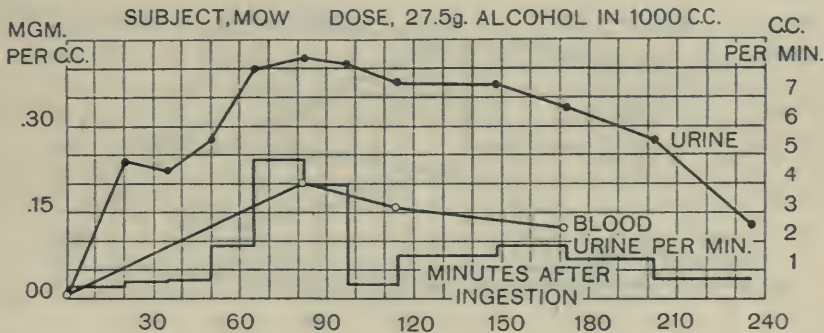


FIG. 2. COMPARATIVE ALCOHOL CONTENT OF BLOOD AND URINE DURING PHYSICAL WORK

ples were passed; it rises quickly; there is an irregularity which cannot be accounted for; it reaches a maximum about eighty minutes after ingestion. The level changes but little sixty-five to one hundred minutes after ingestion, being about at 0.40 mgm. per cubic centimeter. The blood sample taken at 2:31 shows 0.20 mgm. per cubic centimeter, approximately half of what was found in the urine at this point, and corresponds with the highest point in the urine curve. The other two blood samples show smaller values, 0.16 and 0.12 mgm. per cubic centimeter. That the concentration of alcohol in the blood should be so far below the level of that found in the urine was a surprise and it seemed possible that muscular work had disturbed the relationship described by Widmark and others. Since Gréhant (26) had shown that muscular work lowered the alcohol concentration in the blood, and Völtz

and Baudrexel (70) contend that it increases the excretion through the urine, the next experiment was made with the subject quiet, excluding all of the tests and activity that did not have directly to do with the taking of the samples.

Experiment 2. April 21, 1921, on *Mow*. Time of day and other known conditions like the previous experiment. Same dose finished at 1:10. No normal blood sample taken. The subject was quiet except for such muscular exertion as the collection of samples required. The results are given in figure 3. Four blood samples were taken following ingestion by 52, 81, 112, and 171 minutes. The values as seen from the chart are 0.24, 0.28, 0.20, and 0.13 mgm. per cubic centimeter. The corresponding urine values show 0.40, 0.44, 0.43, and 0.30 mgm. per cubic centimeter. Again the blood is only about 50 per cent of the

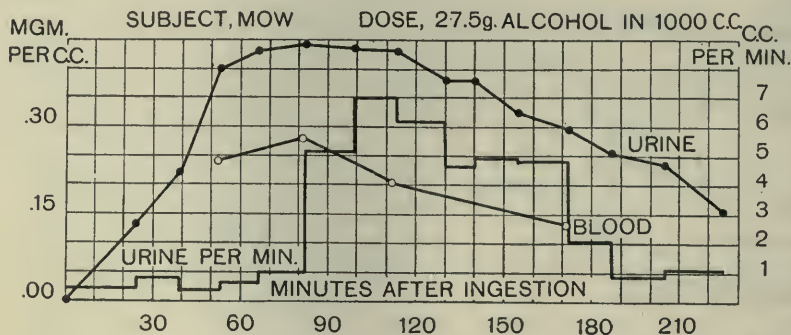


FIG. 3. ALCOHOL CONTENT OF BLOOD AND URINE WITH THE SUBJECT QUIET

values found for the urine. The maxima in general coincide. The total volume of urine passed was 615 cc. as against 364 cc. in the experiment with muscular work. The amount of urine was roughly the same in both experiments up to the point of fifty minutes, where it begins to rise in the case of no. 1. The rise is later but of a larger amount in no. 2, the highest being 7 cc. per minute as compared to 4.8 cc. The alcohol content of the urine is quite independent of the amount of urine passed per minute. The difference in alcohol content between blood and urine found in the two experiments could not be credited as a result of muscular work.

Experiment 3. April 28, 1921, the 27.5 grams of absolute ethyl alcohol were diluted only with water to a volume of 1000 cc. The subject, *Mow*, was in the postabsorptive condition, no food or water having been taken since the time of dinner at 6:30 the previous night. The

dose at 17°C. was ingested between 1:05 and 1:10 p.m. No tests were made in this experiment, the subject remaining quiet between samples. The results are given in figure 4, and agree essentially with those of the previous experiments in the difference between the alcohol content of blood and urine. Portions of the blood samples were in each case centrifuged and determinations made of the concentration of alcohol in the plasma per cubic centimeter. In all the four cases, the plasma shows a higher alcohol content than the whole blood but not as high a content as the urine. Using the average of the four blood determinations as 100 per cent, the plasma is 11 per cent higher and the corresponding urine samples on the average 72 per cent higher. The amount of urine per minute was very small throughout most of the periods, due, of course,

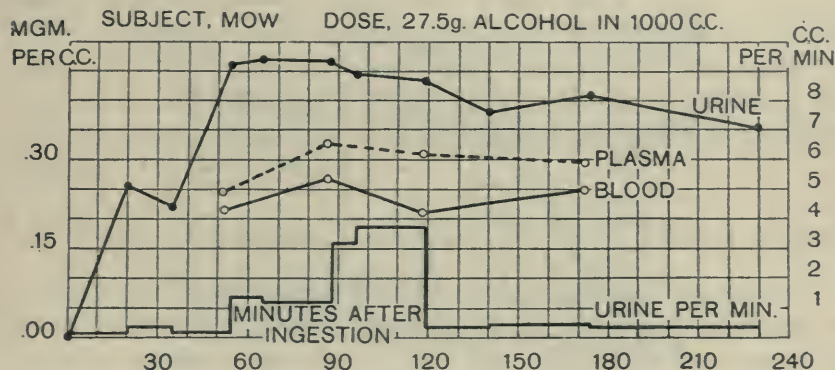


FIG. 4. COMPARATIVE RESULTS FOR URINE, BLOOD, AND PLASMA WITH THE SUBJECT IN THE POST-ABSORPTIVE CONDITION, AND QUIET

The alcohol dose was diluted, with water

to the fact that no liquid other than the dose had been taken since the previous evening. Blood and urine curves decline more slowly from their maxima in this third experiment under the fasting condition.

A 2.75 per cent by weight alcohol solution as used in the three experiments reported is more dilute than was used in those instances where other authors report identity between the alcohol concentrations in blood and urine. The following test was made with the same weight of alcohol but taken in a more concentrated solution.

Experiment 4. May 14, 1921. *Mow* came to the laboratory in the postabsorptive condition but at 7 o'clock a.m. 150 cc. and at 8 o'clock 400 cc. of water was taken. Bladder emptied at 8:45, volume 39 cc.; urine saved as a normal. The 27.5 grams of alcohol were diluted to 50

cc. with water, and 50 cc. of grape juice were added, making a total volume of 100 cc., the percentage of alcohol being 27.5 per cent by weight. Only two blood samples were taken, a large one at 8:30 which was a normal and a sample at 10:00, seventy minutes after the alcohol had been taken, which was at 8:50. The results are shown in figure 5. The amounts of urine obtained within the first hour were larger than previously. The concentration of alcohol in the urine increased promptly and at the end of seventy-two minutes had reached a value of 0.61 mgm. per cubic centimeter. The blood which was taken at this time is also higher than usual, showing 0.47 mgm. per cubic centimeter. The urine is, therefore, about 29 per cent higher than the blood and descends

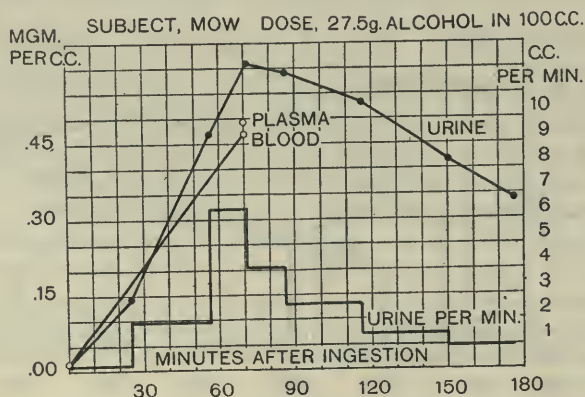


FIG. 5. THE ALCOHOL CONCENTRATION IN BLOOD AND URINE PRODUCED BY A MORE CONCENTRATED BEVERAGE

rapidly. The plasma was slightly higher than the whole blood. In this experiment like the three that preceded this subject does not demonstrate as much alcohol in the blood as in corresponding urine samples.

2. Summary for preliminary experiments 1 to 4, inclusive

(a) From one to two and one-half hours following ingestion of a liter of 2.75 per cent ethyl alcohol by weight, eleven blood samples gave an average of 0.21 mgm. per cubic centimeter while the average alcohol concentration for the corresponding urine samples is found to be 0.40 mgm. per cubic centimeter which is 90 per cent higher than the blood.

(b) This relationship between the alcohol in the blood and urine is not materially modified by muscular work, quiescence, fasting, or the presence of small amounts of food in the stomach.

(c) The amount of urine passed per minute may vary widely and may come to its maximum at different times after ingestion without appreciably changing the contour of the alcohol-urine curve, an observation which substantiates that of Widmark.

(d) There is about 22 per cent more alcohol per cubic centimeter in the plasma than in the whole blood.

(e) The alcohol content of both blood and urine reaches its maximum about the same time, i.e., sixty to ninety minutes after ingestion.

(f) When the same amount of alcohol is taken in a 27.5 per cent solution (exp. 4) the alcohol, especially in the blood, is high than following the 2.75 per cent beverage. The urine percentage-wise is not so far above the blood but still it is definitely higher.

3. Results on a group of abstainers and very moderate drinkers

In order to ascertain whether the results on *Mow* were characteristic or exceptional, and since they were quite contrary to earlier published results, a pair of experiments was made on each of several other subjects. In the literature there is but little comparative data on the alcohol content of both blood and urine samples either with animals or humans. Furthermore, there is very little of any kind of alcohol data which has been secured when using solutions as dilute as 2.75 per cent by weight (1), (60). Since my results in certain respects do not agree with the conclusions of other workers it is necessary to place on record all the experiments so that in so far as possible, the reader may come to an independent judgment in the matter. The experiments are given essentially in chronological order, with a single chart and very brief description devoted to each. The charts are uniform and as the results on the different subjects have very considerable similarity, the descriptive matter is reduced to a minimum.

Experiment 5. May 20, 1921. *Faf*, complete abstainer, age twenty-three years, height 177 cm. weight 70 kilograms, perfect health. Breakfast at 8:00. One glass of water at 10:00 a.m. and at 12:30 p.m. Came to the laboratory at 1:30 p.m., having had no lunch or food since breakfast. After fifty minutes' practice with the tests, a normal blood sample was taken at 2:40 and a urine sample at 2:42. The 1-liter dose was finished at 2:51 and the experiment continued as indicated in figure 6. A blood sample was taken twenty minutes after ingestion, i.e., at 3:11, and the alcohol content, 0.22 mgm. per cubic centimeter, is greater than that for the corresponding urine five minutes after the blood sample. But if the urine value 0.15 were plotted halfway in the period

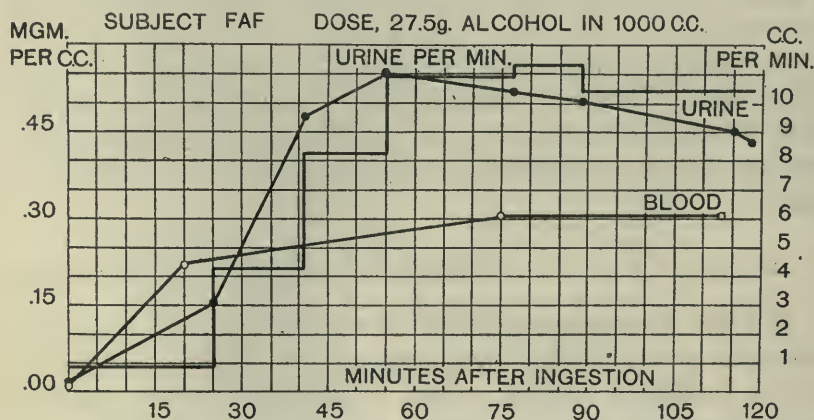


FIG. 6. THE ALCOHOL IN BLOOD AND URINE OF AN "ABSTINENT" PRODUCED BY A 2.75 PER CENT BEVERAGE

which it represents rather than at the time it was taken it would fall on the blood curve. The blood passes to a level of about 0.30 mgm. while the urine at fifty minutes reaches a value of 0.55 mgm. per cubic centimeter, from which it gradually falls, but the blood curve seems to remain on a plateau. The amount of urine per minute is much larger than for *Mow*. The subject thought he had completely emptied the bladder at 4:46, two minutes following the last blood sample, 4:44. He had passed 174 cc. of urine and came back to the table where the blood sample had been taken, but concluded that the bladder was not empty. He passed at this time, three minutes after the previous sample, 129 cc. Duplicate analyses of the 4:46 sample absolutely agreed on 0.455 while duplicate analyses of the 4:49 sample showed 0.429,

which indicates a drop in alcohol content. *Faf* reported and showed slight dizziness and incoördination.

Experiment 6. June 1, 1921, *Faf* with food and physical condition same as before. Began work with tests at 12:30 p.m. Normal blood 1:52, urine 1:54, 288 cc. Both showed good blanks. He drank the regular 100-cc. dose, finishing at 2:04 p.m. The mouth was rinsed out with a small amount of water, as he complained of the taste. Blood sample following ingestion was at 2:25 with urine sample at 2:27. The time intervals and results are given in figure 7. The urine alcohol quickly rises, reaching 0.52 mgm. per cubic centimeter forty-two minutes after ingestion and maintaining this height for about three-quarters of an hour. The urine is only a little higher than the maximum in

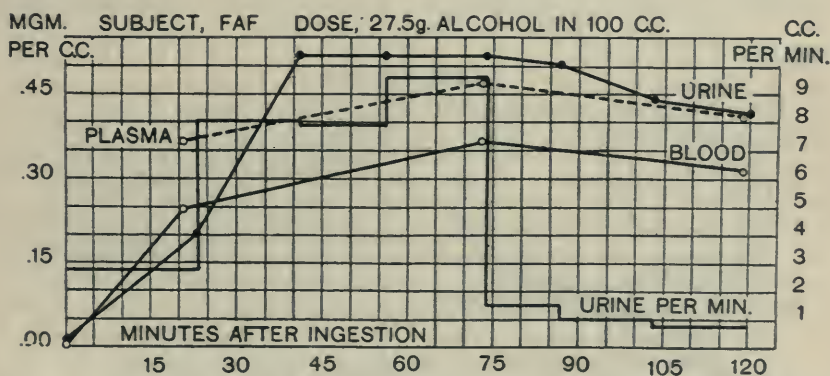


FIG. 7. THE ALCOHOL IN THE BLOOD AND URINE OF AN "ABSTINENT" PRODUCED BY 27.5 PER CENT BEVERAGE

experiment 5 but the blood is 12 per cent higher; thus with the concentrated dose, urine and blood are nearer but still quite apart. The plasma is much above the whole blood and in the latter half of the experiment nearly as high as the urine. The amount of urine per minute is larger with the 100-cc. dose in the forepart of the experiment. He reported subjective symptoms of "daze rather than of dizziness" from twenty until fifty minutes after ingestion. These effects faded a little quicker than in the previous experiment and were of "about the same intensity."

Experiment 7. May 25, 1921, *Kom* reports irregular use of beer, never intoxicated, age twenty-two years, height 181 cm., nude weight 73.4 kgm., excellent health. He came to the laboratory at 1:00 p.m., having had a rather light breakfast and no lunch, one glass of water

taken at breakfast time, no other liquid. At 2:00 p.m. normal urine, 164 cc.; normal blood at 2:03 which gave blanks. Drank the regular liter dose between 2:07 and 2:10. The chart, figure 8, shows a delayed rise in the urine curve probably due to incomplete emptying of bladder twenty-five minutes after ingestion, for the urine per minute is very high in the next period. The highest urine, 0.43 mgm., is 45 per cent above the corresponding blood. There was no plasma available at this point, hence the other plasma values are unconnected. At the end the plasma gives a value identical with the urine in alcohol concen-

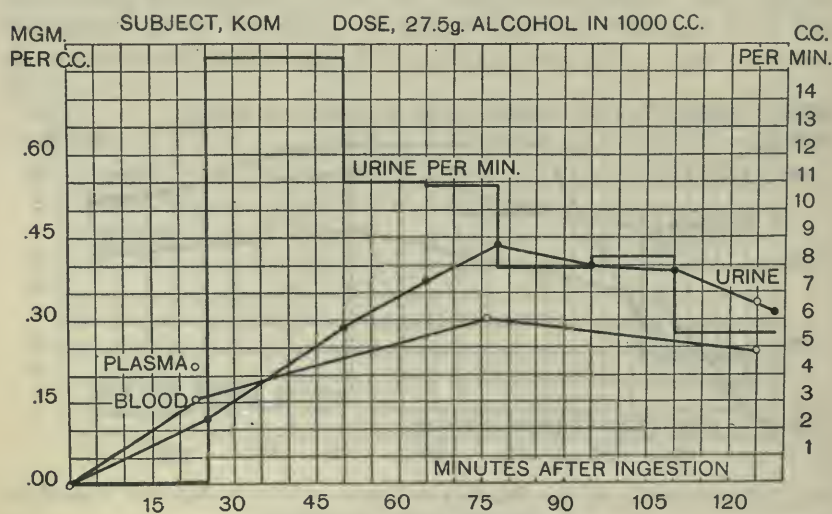


FIG. 8. CONCENTRATION OF ALCOHOL IN THE BLOOD AND URINE OF AN IRREGULAR DRINKER, 2.75 PER CENT DOSE

tration. About twenty minutes after ingestion there was slight dizziness which soon left.

Experiment 8. May 27, 1921, *Kom* with food and physical condition as before. After about one hour of practice with the tests, a normal blood sample was taken at 2:02 p.m. and normal urine at 2:05, volume 34 cc. The urine gave approximately a blank but the blood showed reducing substances equivalent to about 0.04 mgm. per cc. The regular 100 cc. dose was taken at 2:12. A blood sample followed at 2:33 but the subject found it impossible to get a urine sample until 2.43, ten minutes later. The results are plotted in figure 9, which shows the blood to be relatively high, 0.29, at the beginning as compared with 0.21

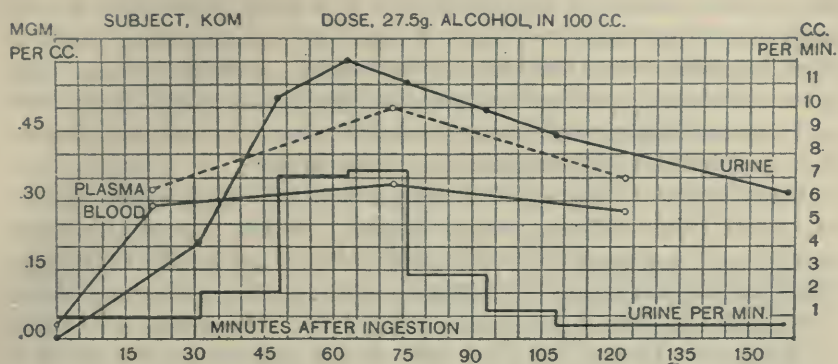


FIG. 9. CONCENTRATION OF ALCOHOL IN THE BLOOD AND URINE OF AN IRREGULAR DRINKER, 27.5 PER CENT DOSE

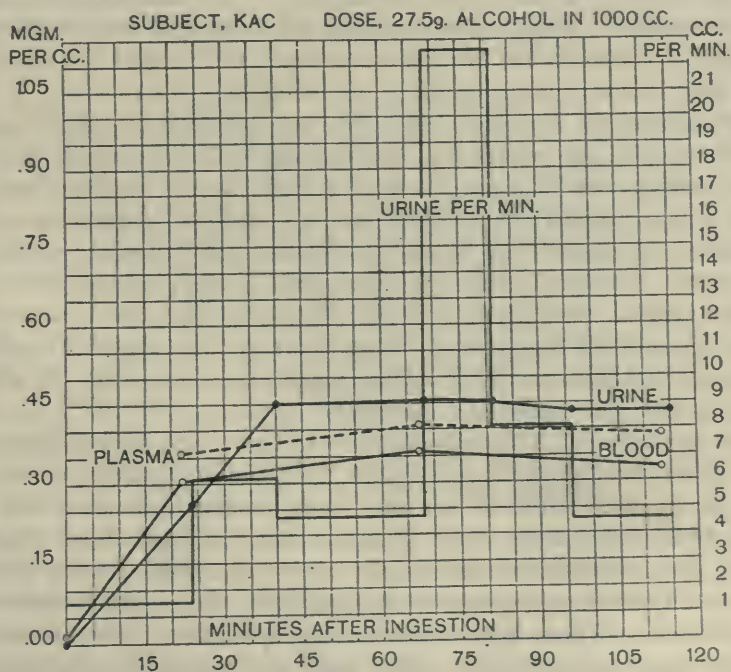


FIG. 10. COMPARATIVE ALCOHOL CONCENTRATION IN BLOOD AND URINE OF A MODERATE DRINKER, 2.75 PER CENT BEVERAGE

in the urine curve ten minutes later. The urine curve rises rapidly from here to its highest value, 0.60 mgm. per cc., sixty-three minutes after ingestion, while the blood remains quite constant at 0.33. The plasma is much higher but not quite as high as the urine. Both urine and blood are higher with the concentrated dose. In both experiments *Kom* had difficulty in passing urine at the first.

Experiment 9. May 31, 1921, *Kac*, a moderate drinker, age twenty-two years, height 175 cm., weight 68 kgm. He had a hearty breakfast at 8:00 a.m., and came to the laboratory without any lunch at 1:00 p.m. The normal blood sample at 2:08 gave 0.015 but the urine was a blank. The regular one liter dose was finished at 2:24. The results are plotted in figure 10. During the later part of the experiment, the urine is about 27 per cent higher than the blood, and 16 per cent higher than the plasma. It is very significant that the greatly increased diuresis which occurred sixty-eight to eighty-one minutes after ingestion (22.5 cc. per minute) produced no fluctuation in the alcohol-urine curve. He reported: "I could easily recognize the alcohol effect and felt some dizziness." Due to the coming of vacation, the subject left the city and there are no results available for the 100-cc. dose.

Experiment 10. June 6, 1921, *Hic*, an abstainer, had never given blood samples, age twenty-four years, height 177 cm., weight 68 kgm., good health. At 8:15 a.m. he had a fairly heavy breakfast and reported at the laboratory without lunch at 12:40. He drank one glass of water and emptied the bladder at 12:50, when he began working on the tests. A normal urine was taken at 2:17, 88 cc. which showed a perfect blank. Heretofore, with the other subjects, a blood sample had been taken before ingestion of the alcohol and three others afterwards. Beginning on this day, *all four blood samples were taken after the alcohol was ingested.* *Hic* finished drinking the 1-liter dose at 2:24. The first blood was taken at 2:44 followed at 2:45 by a urine sample, figure 11. The amounts of alcohol appearing in the blood and plasma at twenty- and forty-minute intervals are quite small as also is the case with the urine.⁷ After about one hour, the urine rises to a higher level than the blood and inscribes a fairly regular curve, the highest point coming one and three-quarter hours after ingestion. The highest blood was the last one. These facts indicate slow absorption. The amount of urine passed per minute was quite small and regularly so for one and one-half hours and the total for two and three-quarters hours was only 582

⁷ The plasma is very near the whole blood and it may be significant that both blood samples were slightly coagulated.

cc. We have in this instance a rather unusual condition. The curves are rather flat compared with those thus far examined except that the third plasma is high, being above the urine. The subject once or twice felt a slight nausea which he thought due to the liquid taken on a relatively empty stomach. There was no dizziness experienced.

Experiment 11. June 8, 1921, *Hic* had a medium breakfast at 8:15 with a glass of water at 9:00 a.m. and again at 11:45. Condition

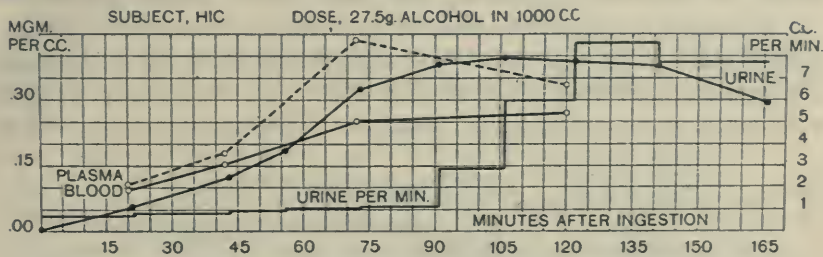


FIG. 11. RELATIVE ALCOHOL CONCENTRATION IN BLOOD AND URINE OF AN OCCASIONAL USER OF WINE, 2.75 PER CENT BEVERAGE

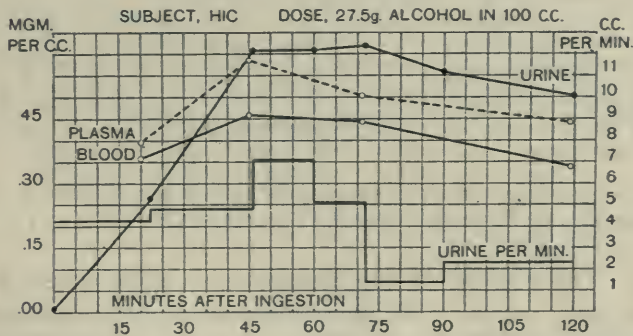


FIG. 12. RELATIVE ALCOHOL CONTENT OF BLOOD AND URINE FOLLOWING THE INGESTION OF 27.5 PER CENT ALCOHOL

reported as normal and unfatigued. Came without lunch at 1:00 p.m. and drank 300 cc. of water, emptying the bladder immediately afterwards. A normal urine sample was taken at 2:00, in volume 410 cc. The regular 100-cc. dose was finished at 2:03. For the results, see figure 12, which is quite in contrast with the former experiment. The urine, blood, and plasma curves rise promptly, reaching the highest point about forty-five minutes after ingestion. Here the plasma and urine are about equal and the latter is approximately 30

per cent higher than the blood. The urine per minute is large from the first. A prominent alcohol effect was reported present beginning ten minutes after drinking and continuing a short while. No headache or nausea were present.

Experiment 12. June 13, 1921, *Jem*, usually an abstainer, age twenty-five years, height 172 cm., weight 63 kgm., good physical condition. Having had a hearty dinner the night before at 7:00 p.m., he drank two glasses of water in the morning and came to the laboratory at 8:30 a.m. without breakfast. The bladder was emptied and work with the tests begun. A normal urine was collected at 9:27, 42 cc. volume. The regular 1-liter dose was finished at 9:33. Blood and urine samples were taken at about the usual intervals. The results are charted in

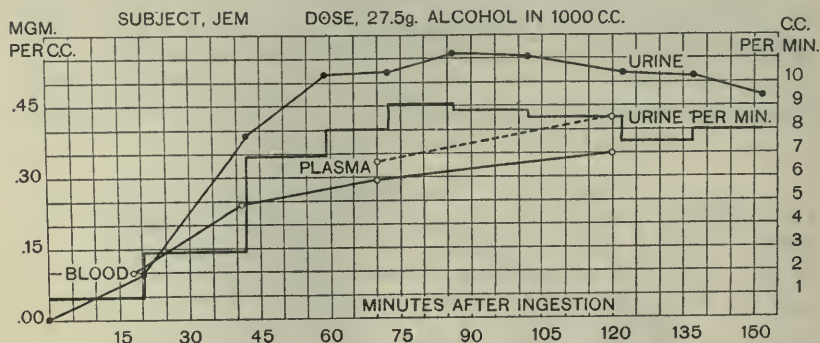


FIG. 13. COMPARATIVE ALCOHOL CONTENT OF BLOOD AND URINE OF AN OCCASIONAL DRINKER IN POST-ABSORPTIVE CONDITION, 2.75 PER CENT BEVERAGE

figure 13. The plateau of the alcohol-urine curve which averages about 0.54 mgm. per cc. is 70 per cent higher than the average for the last two bloods and 43 per cent higher than the average for the last two plasmas. A little dizziness and slight nausea were experienced, beginning fifteen minutes after ingestion and lasting until the last blood sample.

Experiment 13. June 14, 1921, *Jem* had a hearty breakfast at 8:55 a.m. with a glass of water at each of the following times: 8:55, 11:45, 12:20, and in the laboratory at 1:00. No lunch or food following the breakfast. Normal urine at 1:56, volume 65 cc. The regular 100-cc. dose finished at 1:58. The results are given in figure 14. In general, they show higher values at all points than in the previous experiment. The curves in each case also reach a definite peak at a somewhat earlier

time, about seventy minutes following ingestion. The relationship between the concentration of alcohol in the blood, plasma, and urine agrees with the previous experiments. He reported no nausea and no tendency to regurgitate; dizziness and a peculiar selfconfidence were present. "If I make a mistake, it does not seem to worry me."

Experiment 14. June 16, 1921, *Sul*, moderate drinker, had never given blood samples, once drank 2 liters of liquid within a short while without discomfort, age twenty-nine years, height 170 cm., weight 67 kgm., good health. Came to laboratory at 8:30 a.m. *without breakfast* but having taken about 400 cc. of water at 7:45 a.m. The bladder was emptied at 8:30. No water was drunk at the laboratory. "Nor-

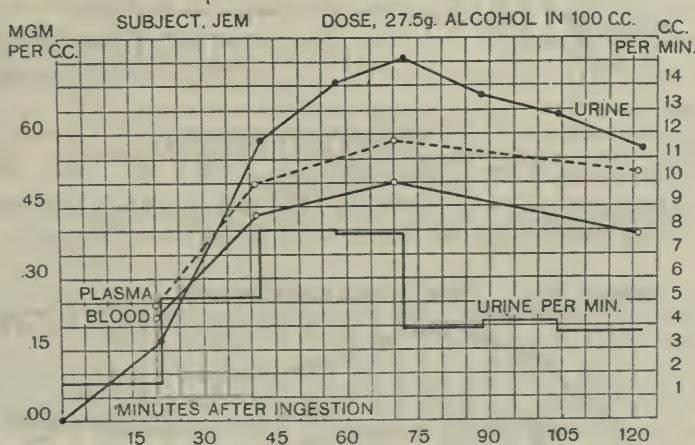


FIG. 14. COMPARATIVE ALCOHOL CONTENT OF BLOOD AND URINE IN AN OCCASIONAL DRINKER FOLLOWING THE INGESTION OF 27.5 PER CENT BEVERAGE

mal urine" passed at 9:33, volume 36 cc. By mistake this urine was not taken before the subject began to drink. After he had ingested half of the regular 1-liter dose, it was recalled that he had not emptied the bladder just before. This urine, therefore, comes about three minutes after he began to drink. The dose was finished at 9:40. The first urine is higher than the usual blank as it shows 0.026 (figure 15). The first blood sample is twenty-seven minutes after ingestion due to the unavoidable absence of the assisting doctor. Its alcohol content is below that of the urine. From this point the urine curve rises definitely above the blood and plasma curves, about 70 and 27 per cent respectively. The amount of urine per minute was quite large. The

subject was observed to be unsteady in his gait and to show some confusion.

Experiment 15. June 20, 1921, *Sul* came to the laboratory at 8:40 without breakfast having taken two glasses of water. At 8:45, he drank

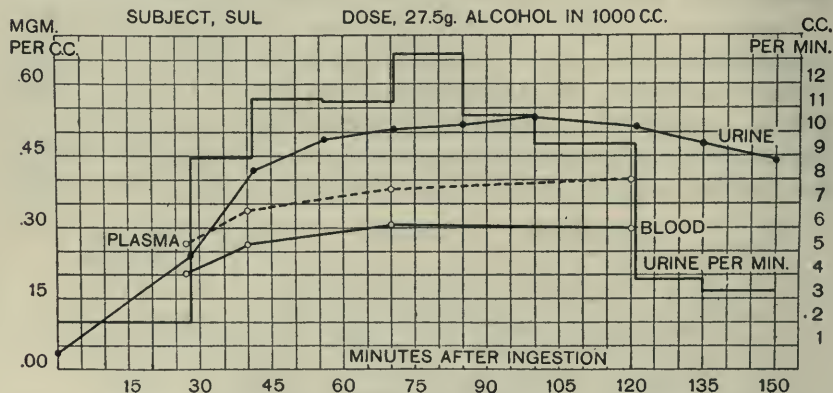


FIG. 15. ALCOHOL CONTENT OF THE BLOOD AND URINE OF A MODERATE DRINKER IN THE POST-ABSORPTIVE CONDITION, 2.75 PER CENT BEVERAGE

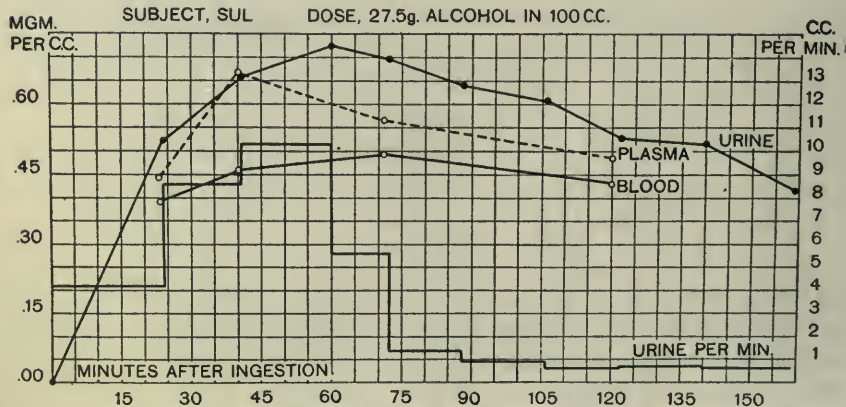


FIG. 16. ALCOHOL CONTENT OF THE BLOOD AND URINE IN A MODERATE DRINKER IN THE POST-ABSORPTIVE CONDITION, 2.75 PER CENT BEVERAGE

one glass of water and emptied the bladder at 9:10. Normal urine saved at 10:00, 355 cc. Drank the regular concentrated dose of 100 cc., volume at 10:02. The results, given in figure 16, show higher urine and blood alcohol values than were found with the dilute dose, also

more definite and earlier maxima in the curves. The plasma found with the second blood sample seems extraordinarily high, otherwise the blood and plasma determinations are all definitely lower in alcohol concentration than is the urine. The subject showed typical alcohol symptoms which took the form of a jolly exuberance, and clumsy movements which apparently he made no effort to inhibit.

Experiment 16. July 5, 1921, *Rir*, moderate drinker, age thirty-three years, height 174 cm., weight 75 kgm., excellent health. The previous day, July 4, was spent entirely in strenuous exercise, playing tennis, sailing, walking, swimming, etc., also an unusually large amount of food was taken. He ordinarily urinates about 900 cc. in twenty-four hours as found from four different tests. He had a medium breakfast at 7:30 but no water with his breakfast. Thinking that his system might be abnormal because of the large amount of exercise on the

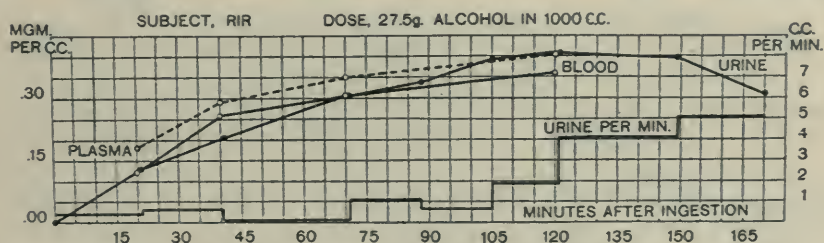


FIG. 17. ALCOHOL IN THE BLOOD AND URINE OF A SUBJECT WHO, ON THE DAY PREVIOUS, HAD ENGAGED IN A GREAT AMOUNT OF PHYSICAL EXERCISE WITH THE RESULT THAT HE WAS DEFICIENT IN WATER, 2.75 PER CENT DOSE

previous day, he drank three glasses of water between the hours of 7:30 and 10:00 and came to the laboratory without lunch at 1:00 p.m.; the bladder was then emptied. Normal urine passed at 1:50, 40 cc. The regular 1-liter dose was consumed, finishing at 2:00. The results of the experiment are shown in figure 17, the striking feature of which is the close agreement between the curves for the blood, plasma, and urine. They not only run quite parallel with one another but are quite close to the same value. The amounts of alcohol for blood and plasma are about as high as usually found for the 1-liter dose. The urine curve compared with others is on the contrary decidedly depressed. In connection with these facts, it is noticed that the amount of urine passed per minute during the first two hours after ingestion averaged only about 0.5 cc. which is very small indeed considering the amounts of liquid ingested. It was possible to repeat this experiment when the subject was in a more normal condition of water balance.

Experiment 17. July 19, 1921, *Rir* had no heavy exercise on the previous day, physical condition good, breakfast at 7:30, two glasses of water taken during the morning. He came to the laboratory at 1:05 p.m. without lunch and drank two glasses of water and emptied the bladder. Normal urine passed at 1:35, 25 cc. Regular 1-liter dose finished at 1:45. The data of this experiment, which closely resemble results with other men, are plotted in figure 18 and show a definite difference from the previous experiment in the early concentration of alcohol appearing in the urine. The amount of alcohol in the blood is about the same in both cases for the first 3 samples. On July 5, the last sample is the highest of all, amounting to 0.36, whereas on July 19 the last is 0.26 mgm. per cc. The amount of urine per minute in the experiment of July 19 is very much larger than in the former one but not as high as the average with other subjects. In

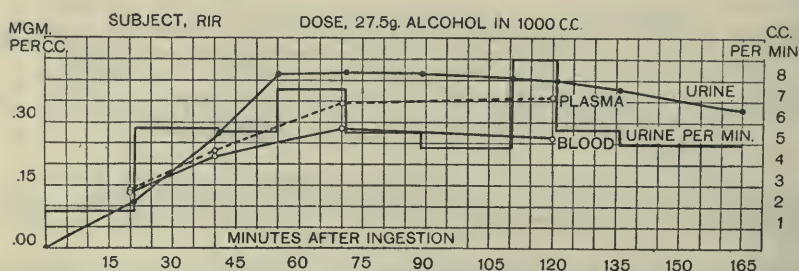


FIG. 18. ALCOHOL IN THE BLOOD AND URINE WHEN THE BODY IS IN A MORE NORMAL CONDITION OF WATER BALANCE, 2.75 PER CENT DOSE

both these experiments the subject reported hardly any trace of dizziness or vertigo.

Experiment 18. July 6, 1921, *Rir* had breakfast at 8:00 a.m. with one cup of cereal coffee and three glasses of water during the forenoon, also one glass at 1:00 p.m. after coming to the laboratory, when the bladder was emptied after drinking. Normal urine at 1:59 p.m., 76 cc. Regular concentrated dose of 100 cc. finished at 2:00. The blood and urine samples were taken at the usual intervals as shown in figure 19. The amounts of alcohol in the blood, and particularly in the plasma, are higher than in the corresponding urines twenty and forty minutes after ingestion, as plotted, but virtually about the same if the urine values are averages for their respective periods. Then the blood reaches 0.48 while the urine reaches 0.64, which is 33 per cent above it. The plasma is but little lower than the urine. All curves reach an earlier

and more definite peak. The total amount of urine secreted during the experiment was 302 cc.; this is less than the average with the concentrated dose. For about one hour after taking the alcohol the subject reported drowsiness which he attributed to it.

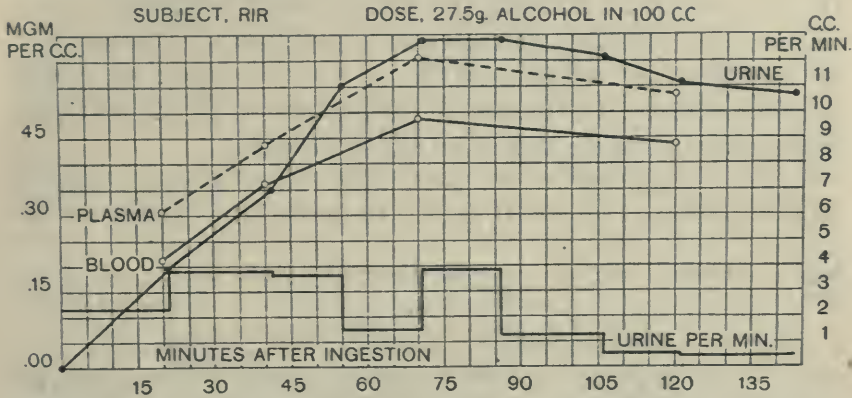


FIG. 19. ALCOHOL IN THE BLOOD AND URINE FOLLOWING THE 27.5 PER CENT BEVERAGE

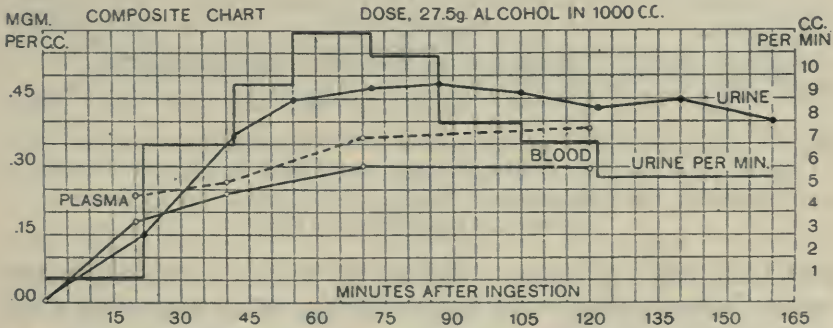


FIG. 20. TYPICAL RESULTS ON ABSTINENT AND MODERATE DRINKERS FOR THE COMPARATIVE CONCENTRATION OF ALCOHOL IN URINE, BLOOD, AND PLASMA FOLLOWING THE INGESTION OF A LITER OF 2.75 PER CENT BY WEIGHT ALCOHOL BEVERAGE, TAKEN WITHOUT FOOD

4. Summary for experiments 5 to 18, inclusive

The foregoing subjects as a group have all been abstinent or quite moderate in their use of alcohol. The results are in general so consistent from man to man as to admit of presentation in a composite chart for each alcohol dose used. Figure 20 is such

a composite for the results obtained on six different men after the ingestion of 1 liter of 2.75 per cent ethyl alcohol by weight. The six experiments so combined are nos. 5, 7, 9, 12, 14, and 17. Similarly figure 21 is a composite for six experiments, nos. 6, 8, 11, 13, 15, and 18, showing the concentration of alcohol in the venous blood, plasma and urine also the amount of urine secreted per minute after ingesting 100 cc. of a 27.5 per cent by weight alcohol solution. In all cases the alcohol was taken diluted with grape juice and water and on an empty stomach.

(a) The composite chart for 27.5 grams of alcohol taken in 1000 cc. shows, at 20, 40, 70, and 120 minutes after ingestion 0.18,

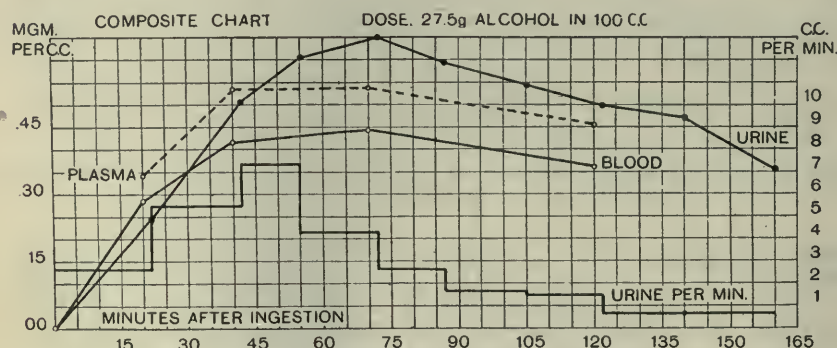


FIG. 21. TYPICAL RESULTS ON ABSTINENT AND MODERATE DRINKERS FOR THE COMPARATIVE ALCOHOL CONCENTRATION IN URINE, BLOOD, AND PLASMA FOLLOWING THE INGESTION OF 100 CC. OF 27.5 PER CENT BY WEIGHT ALCOHOL, TAKEN WITHOUT FOOD

0.24, 0.30 and 0.30 mgm. of alcohol per cubic centimeter in the whole venous blood. The corresponding urine values are 0.15, 0.37, 0.47, and 0.43 mgm. per cubic centimeter indicating a higher concentration of alcohol in the urine by approximately 50 per cent from forty minutes to two hours after ingestion, and also higher than the plasma by about 25 per cent during the same period.

(b) The composite chart for 27.5 grams of alcohol taken in 100 cc., figure 21, shows 0.28, 0.42, 0.43, and 0.36 mgm. of alcohol per cubic centimeter in the blood and at the corresponding intervals 0.24, 0.51, 0.65, and 0.49 mgm. in the urine. The urine

is therefore 37 per cent higher than the blood in alcohol concentration forty to one hundred and twenty minutes after ingestion, but as an average for the same period only 8 per cent higher than the plasma.

(c) The same amount of alcohol taken in a more concentrated form (27.5 as compared to 2.75 per cent by weight) gave higher concentration of alcohol in blood, plasma, and urine. The increase over the results with the dilute dose are 47 per cent and 50 per cent for blood and plasma, while the corresponding urine samples are 33 per cent higher. Comparing the two composite charts, point for point, as if superimposed there is only one instance, the alcohol-urine one hundred and sixty minutes after ingestion, where the dilute dose gave as high or a higher alcohol concentration.

(d) The alcohol concentration in blood and urine reaches its maximum sixty to seventy-five minutes after ingestion. The maximum is prolonged for thirty to forty-five minutes with the dilute dose. On the contrary it is sharp and followed by a definite decline in the curves when the alcohol has been taken in concentrated form.

(e) The alcohol concentration of the blood is for the first forty minutes quite near that of the urine but seventy minutes following ingestion, the urine departs most widely from it, showing an alcohol content 30 to 50 per cent higher.

(f) The alcohol concentration in the plasma is 20 to 25 per cent higher than that in the whole blood. For about forty minutes after the ingestion of alcohol the concentration is as high in the plasma as in the urine, later the urine is consistently higher.

(g) When the kidney secretion is slight, i.e., with very small urine volumes, (exp. 10 and 16) the alcohol concentration in the urine may be reduced to approximately the same level as that of the blood.

(h) The amounts of urine per minute were naturally larger with the liter dose. In each composite chart, this block curve reaches its crest about simultaneously with the alcohol-urine curve, but individual experiments show that the even contour of the alcohol-urine curve is not disturbed by rather wide changes in diuresis.

(i) These results are in agreement with those for experiments 1 to 4 on *Mow* and show that he was not an exceptional case but that actually for the experimental conditions used the alcohol content of venous blood and urine do not wholly agree or parallel with each other in the two hours following ingestion.

5. Results on men who habitually or occasionally drink much alcohol

Experiment 19. July 13, 1921, *Cef* is an habitual drinker of long experience, age fifty-two years, height 177 cm., weight 65 kgm. Breakfast at 8:00 a.m. Morning spent in walking, drank some spring water, quantity unknown. Came to laboratory at 1:00 p.m. without lunch

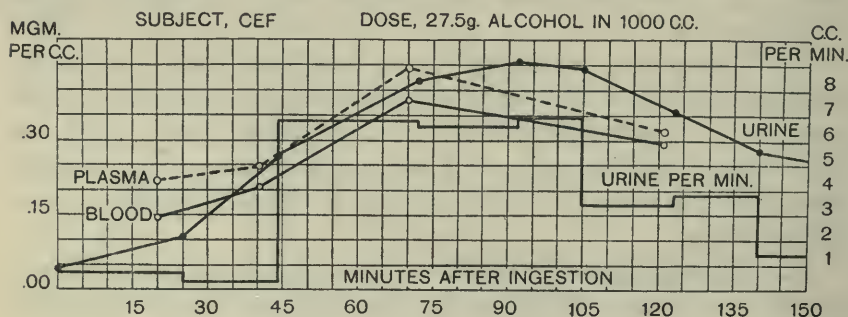


FIG. 22. ALCOHOL IN THE BLOOD AND URINE OF AN HABITUAL DRINKER AFTER TAKING 1 LITER OF 2.75 PER CENT BEVERAGE

and claimed to be quite normal and to have had no alcohol within the previous twenty-four hours. Normal urine taken at 1:51, 56 cc. As will be seen in figure 22, it was not blank. The regular 1-liter dose was finished at 2:00. Blood and urine samples were taken as usual. The amount of alcohol appearing in the blood is about the same as the average with other subjects although the third sample seems to present a more definite and higher peak than is usually found. The blood is below the urine in alcohol content. The urine appears to reach its highest point about ninety minutes after ingestion. The subject could not pass urine at fifty-five minutes and so waited until following the third blood sample. The urine reaches an alcohol content of 0.46 mgm. per cubic centimeter, which agrees well with that found with the others but the curve has a sharper downward trend. Concerning the

experiment, *Cef* remarked: "What I drank today tasted well, volume not large if you are thirsty; would hardly know it contained any alcohol."

Experiment 20. July 15, 1921, *Cef* had breakfast of medium amount at 8:15 a.m. "Not a thing with alcohol in it since the former experiment." Came to the laboratory without lunch at 12:45 p.m. Drank 400 cc. of water at 1:00, and emptied the bladder. Normal urine taken at 1:57, 202 cc. This is not blank as it shows 0.045 mgm. reducing substance per cubic centimeter. Drank the regular concentrated dose at 2:00. The results which are plotted in figure 23 are different than any previously shown. By twenty minutes after in-

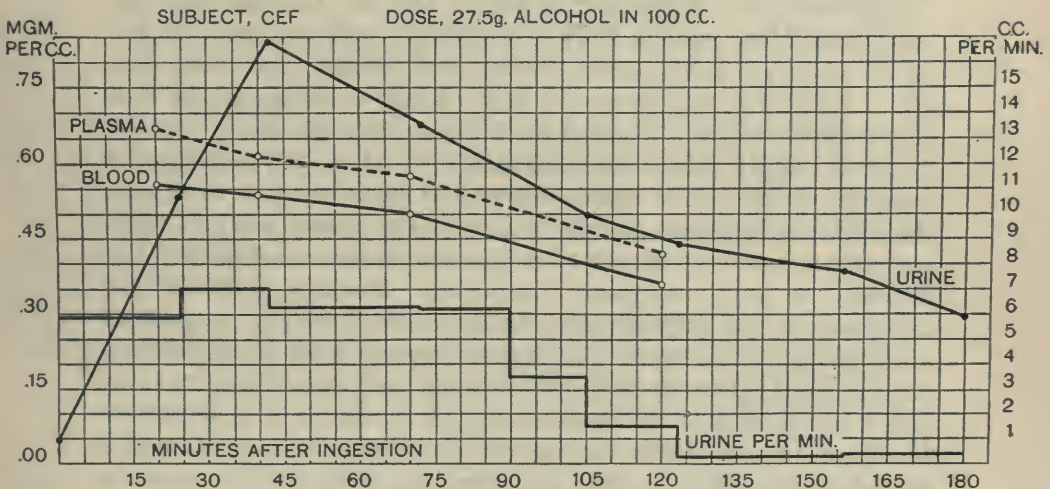


FIG. 23. ALCOHOL IN THE BLOOD AND URINE OF AN HABITUAL DRINKER AFTER TAKING 27.5 PER CENT BEVERAGE

gestion the alcohol had reached a content of 0.56 in the blood and of 0.67 mgm. per cubic centimeter in the plasma. These are higher values than any other subject showed at any time with the concentrated dose. From this twenty-minute point both the blood and plasma curves gradually decline; in fact, the curves suggest those obtained by Gabbe (19) when the alcohol was injected directly into the blood stream. The alcohol-urine curve rises sharply and forty-two minutes after ingestion reaches the surprising value of 0.84 mgm. per cubic centimeter, from which it immediately and continuously declines. The diuresis was large (37) and double sets of duplicate analyses leave no room to doubt the alcohol content of the specimens.

The comments of this habitual subject concerning the concentrated dose are as follows: "Could feel the dose inside of me within two minutes, not enough to start me talking but another one the same size would have started me. Strongest effects about twenty minutes after the drink and did not get any worse but tapered off from then on. No wobbly feeling in legs, or dizziness; just a good feeling, the kind you spend money for; you can feel a heat all over the body resulting from it. The drink made no difference on my work with the tests but helped me out in sitting quiet and stuff of that kind, could stand steadier with it than without it."

Experiment 21. September 21, 1921, *Haj*, an habitual who had never given blood samples but was certain he would not mind doing so for he said "I'm used to cuts, bruises, and burns." Age thirty-nine years, height 174 cm., weight 69 kgm. Came to laboratory at 8:20 a.m. *without breakfast*. Normal urine taken at 10:05, 41 cc. Subject drank the regular 1-liter dose, finishing at 10:11, and 20 minutes after ingestion, the first blood sample showed 0.31 mgm. per cubic centimeter the corresponding urine sample 0.29, while the plasma was 0.37 mgm. per cubic centimeter. The blood sample was taken without the slightest difficulty or any unnecessary discomfort. However, following the urine sample, the subject stated his refusal to have any more blood samples taken and in spite of all types of argument left the laboratory. A urine sample at 10:55 gave a value of 0.49 mgm. per cubic centimeter. The fragment of data which was secured is consistent in showing both blood and plasma higher than the urine at the twenty minute period and all these values are quite high compared with those in composite chart, figure 20.

Experiment 22. September 27, 1921, *Sut*, an excessive occasional drinker, has the reputation of being able to drink without intoxication. Age twenty-four years, height 169 cm., weight 53 kgm. At 12:45 p.m. a light lunch. Came to the laboratory at 3:20. Normal urine sample passed at 3:24, 110 cc. Took the regular 1-liter dose, finishing at 3:30. Blood and urine samples are shown with results in figure 24. After forty minutes the urine curve rises above those for blood and plasma and slowly reaches a maximum 90 to 105 minutes after ingestion; the blood is highest in the last sample, two hours after ingestion. The chart is not dissimilar to those on abstinent subjects. Urine per minute was reasonably large but reached its highest value more slowly than usual.

Experiment 23. September 30, 1921, *Sut* reports a larger breakfast than usual with one cup of coffee. He had also a large sandwich and one cup of coffee at 12:30 p.m. and drank 250 to 300 cc. of water at 1:40 p.m. "No alcohol since previous experiment." Came to the laboratory at 2:30 p.m. and without the usual practice on the tests before ingestion of the alcohol a normal urine was passed, 216 cc. at 2:33, and the regular 100-cc. alcohol dose was finished at 2:35. The

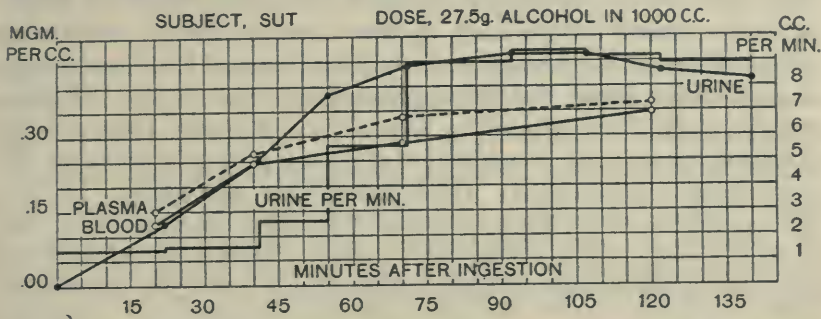


FIG. 24. AMOUNT OF ALCOHOL IN THE BLOOD AND URINE OF AN "EXCESSIVE OCCASIONAL USER" AFTER TAKING 2.75 PER CENT BEVERAGE

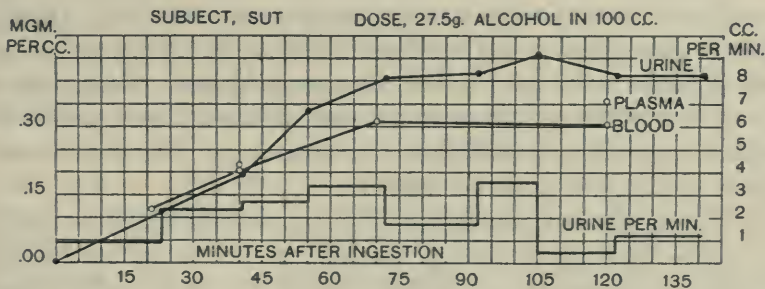


FIG. 25. AMOUNT OF ALCOHOL IN THE BLOOD AND URINE OF AN "EXCESSIVE OCCASIONAL USER" AFTER TAKING 27.5 PER CENT BEVERAGE

results are charted in figure 25. The blood samples were all small, due to the fact that a small syringe had to be used in place of the regular one, which accidentally was broken. The blood and urine results shown in this chart are very similar in alcohol content to those in figure 24 with the more dilute dose; in fact, they are actually the lower. There are only the minor exceptions that the third blood sample is 0.31 as compared to 0.28 and that the three last urines are of about the same alcohol content with both concentrated and dilute doses. This is the

only such instance in nine pairs of experiments. He shows results comparable with the average of the others with the 2.75 per cent beverage, therefore some unusual condition may have been present in the alimentary tract on this occasion when the concentrated dose was taken. It may be significant that he reported having taken more food than usual during the morning. At another time he reported having found a way whereby he could drink considerable alcohol without experiencing strong effects; this was by taking half a glass of olive oil shortly before the alcohol. He is likely correct in this, as Hanzlik and Collins (31) working on the absorption of alcohol introduced into ligated loops of cat intestine found that the introduction of 2 cc. of olive oil into a loop decreased the absorption by 27 per cent. Probably his reputation as a drinker rests on the combination of the use of such tricks along with a constitutional peculiarity which favors slow absorption. He said that no alcohol effects were experienced in either experiment but that in both he was certain alcohol was taken at the time of drinking and later, due to the sensations in the mucous membrane of the mouth (28).

6. Summary for experiments 19 to 23

It is difficult to secure coöperative trustworthy subjects who are really habitually or frequently heavy users of alcohol. Irregularities in the experimental routine seem more likely to occur when working with such men. The five experiments which fall in this class supplement those made on abstinent and moderate drinkers and were undertaken to discover if the "habitual" showed a different type of results.

(a) The alcohol concentration in blood and in urine after taking 27.5 grams whether diluted in 1000 cc. or 100 cc. of fluid is not identical and does not run parallel in the two hours after ingestion. At first, the urine has about the alcohol content of the blood but after forty to sixty minutes it becomes higher than the blood and remains so for at least an hour.

(b) The plasma contains more alcohol than the whole blood, at first slightly more and later considerably more (results with the 2.75 per cent dose).

(c) In the typical "habitual" the alcohol concentration in urine, blood, and plasma is higher at every point with the more concentrated dose.

(d) The above general conclusions are in agreement with the results on abstinent and moderate drinkers.

7. Alcohol concentration in urine on incompletely emptying the bladder

The alcohol concentration in the blood after a single ingestion is constantly changing, unless the rate of absorption varies or just equals that at which the blood gives alcohol to other tissues, a condition called the "Gréhant plateau." A blood sample practically represents a point as it is collected from the general circulation during about one-half minute. Without catheterization, a urine sample represents a period of secretion when probably the alcohol coming to the kidney has varied from moment to moment, and the diuresis has probably not been constant.

In the foregoing experiments, subjects were not catheterized but voluntarily emptied the bladder. This was not done more frequently than about every fifteen minutes. In certain instances, it seemed obvious that some urine was retained in the bladder. To discover the influence that such a condition may have on the alcohol-urine curve was the object of the following test.

Experiment 24. July 27, 1921, *Mow*, used in experiments 1 to 4, was in usual normal condition and had had a medium breakfast at 7:40 a.m. with considerable liquid, one glass of water at 10:00 and at 11:20 two small crackers, one-half of a banana, and two glasses of water, about 400 cc. Just prior to drinking the 400 cc., the bladder was emptied. From then on the subject was to pass urine samples as frequently as possible but in each instance to incompletely empty the bladder. At 12:50, 18 cc. and at 12:55, 17 cc. of urine were passed. At 1:01 to 1:05, i.e., one and three-quarters hours after his light lunch, he drank the regular 1-liter dose of 27.5 grams of absolute alcohol at a temperature of 22°C. The results are given in figure 26, of which *A* shows the alcohol concentration in the urine and indicates the frequency with which samples were passed. The dots marked with a *v* are cases when, judged subjectively, the bladder was *completely* emptied; those inclosed within a circle, when it was *nearly* emptied; all other dots represent *incomplete* urination. Curve *E* charts the amount of urine passed per minute. For comparison, three other alcohol-urine curves for other experiments (same alcohol dose) on this subject are included. In these the bladder was *emptied* at each sample. *B* is the average of a pair of tests when samples were taken every fifteen minutes. The

quantity of food and elapsed time until drinking were the same as in A. Curve *C* is the average of two experiments with thirty-minute samples, the food was the same but the time before drinking was two hours. *D* is the average for experiments 1 and 3 of this paper.

In contrast to *B*, *C*, and *D*, curve *A* shows a delayed rise, depression of maximum, and an extended plateau with slight fluctuations around 0.34 mgm. per cubic centimeter. At one hundred fifty minutes after ingestion the bladder was emptied, 178 cc. of urine being passed, with an alcohol content of 0.345 mgm. A sample of 7 cc., five minutes later, showed a drop to 0.327 mgm. The experiments with fifteen-minute samples, *B*, gave a slightly quicker rise to maxima, higher maxima, and more prompt decline than those, *C*, where thirty-minute samples were

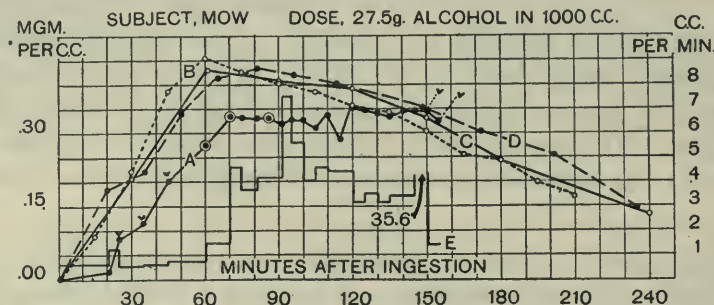


FIG. 26. THE ALCOHOL CONCENTRATION IN THE URINE AS INFLUENCED BY THE RETENTION OF URINE AND FREQUENT PARTIAL EMPTYING OF THE BLADDER

A, incomplete and frequent urination with volumes shown in *E*; *B*, complete urination each fifteen minutes; *C*, and *D*, urination each thirty minutes.

taken. Just as alcohol injected into the blood passes into the stomach, Gréhan (25), so Völtz, Baudrexel, and Deitrich (71), and Nicloux and Norwicka (56) have demonstrated that alcohol placed in the bladder is diffused through the bladder wall into the general circulation provided the alcohol content of the blood is lower than that in the bladder. It has also been found, Völtz (71), that when concentrated alcohol solutions mixed with meat were given to dogs, by frequently rinsing out the bladder the amount of excreted alcohol could be increased three or four times. The diffusion of alcohol through the bladder wall itself thus theoretically influences the alcohol content of the urine excreted, increasing it when the blood is the higher and decreasing it when the urine is higher than the blood. How much of a rôle this factor plays is undetermined. At least it does not operate with sufficient intensity

or promptness to bring the urine into complete agreement with the blood. Retention of urine in the bladder flattens the alcohol curve and the value obtained for any sample represents something of an average for the period and for the total amount of urine then in the bladder.

The reader will have observed that in the foregoing charts each urine value has been plotted at that point on the abscissa when the sample was passed just as with the blood samples. In reality, the alcohol-urine curve theoretically should be shifted somewhat to the left in each figure. If this is done the comparison which the charts show between blood and urine would not be fundamentally altered except that the urine would then be above the blood and almost exactly at the value of the plasma during the first thirty minutes, i.e., the urine and blood curves would not cross.

8. Amount of alcohol excreted in the urine within 2 hours after ingestion

In the individual experiments the urine per minute has been given in the charts. The results in terms of total urine excreted within two hours following ingestion are summarized in table 2 which covers experiments 5 to 23 with the exception of no. 21. There are ten cases when 27.5 grams of alcohol were taken in 1000 cc. These show an average, 669 cc. of urine (37), containing a total of 286 mgm. alcohol which is equal to 1 per cent of the amount ingested and to an average of 0.43 mgm. per cc. The eight experiments with 27.5 grams alcohol taken in 100 cc. give an average total urine of 468 cc., containing 260 mgm. alcohol, which amounts to 0.9 per cent of the quantity ingested and shows 0.55 mgm. per cubic centimeter as the average alcohol content of the urine. The high alcohol concentration in the urine with the 100-cc. dose to some extent counterbalances the larger urinary volume following the 1000-cc. ingestion so that the per cent of alcohol excreted is not far different. However, most of the subjects, even though a fairly large extra quantity of liquid was given prior to the 100-cc. dose so that urine samples would be insured, show a larger percentage of elimination following the dilute dose. The average for this, 1.0 per cent, is considerably influenced by experiments 10 and 16 where the subjects passed but little urine during the two hours. Omitting these, the average

elimination would be 1.2 per cent, which more nearly agrees with the 1.4 per cent found on an average in fourteen other experiments not here reported where the 1000 cc. dose was used with these same subjects.

It is judged from the work of Higgins (35), Völtz and Dietrich (73) (75), and Batelli and Stern (4) among others, that 0.5 cc. of absolute alcohol per kilogram of body weight given in one portion will require about eight hours to disappear from the body. Seppä (64) experimenting with 50-cc. doses on himself,

TABLE 2

The total urine excreted within two hours after ingestion, showing the total and per cent of alcohol eliminated in this period

SUBJECT	DILUTE DOSE 27.5 GRAMS ALCOHOL IN 1000 CC.				CONCENTRATED DOSE 27.5 GRAMS ALCOHOL IN 100 CC.			
	Number and date	Total urine	Total alco- hol	Per cent ex- creted	Number and date	Total urine	Total alco- hol	Per cent ex- creted
		cc.	mgm.			cc.	mgm.	
Faf.....	5, May 20	891	427	1.5	6, June 1	577	267	1.0
Kom.....	7, May 25	1058	368	1.3	8, May 27	338	184	0.7
Kac.....	9, May 31	802	345	1.2				
Hic.....	10, June 6	227	73	0.3	11, June 8	476	246	0.9
Jem.....	12, June 13	743	378	1.4	13, June 14	578	367	1.3
Sul.....	14, June 16	1061	514	1.9	15, June 20	565	370	1.3
Rir.....	16, July 5	86	27	0.1	18, July 6	292	136	0.5
Rir.....	17, July 19	662	241	0.9				
Cef.....	19, July 13	500	204	0.7	20, July 15	665	420	1.5
Sut.....	22, Sept. 27	664	278	1.0	23, Sept. 30	249	86	0.3
Average.....		669	286	1.0		468	260	0.9

taken in 40 per cent solution, found the urine to be alcohol-free after approximately seven hours. This agrees with determinations which we have made on *Mow* who took the 1000-cc. dose without food and collected urine samples, at first each thirty minutes and later each one or two hours. The average for four such experiments not otherwise reported in this paper in which about 29 grams of alcohol were taken in 1000 cc. shows that after eight hours, the urine is practically alcohol free. The total volume of urine averaged 1,340 cc. and contained 1.86 per cent

of the alcohol quantity ingested. During the first two and one-quarter hours, 958 cc. of urine were passed and this contained 1.55 per cent of the alcohol, while the 384 cc. of urine excreted from two and one-quarter to eight hours after drinking contained only 0.31 per cent. Somewhat more than 80 per cent of the excreted alcohol appears within two hours, providing the bladder is emptied two or three times within this period. This result is in agreement with Haneborg (29), page 26), who places the two-hour figure at 90 per cent of the excreted alcohol.

Since 1877, when Binz (7) and Heubach (33) worked on the problem of elimination, it has been recognized that the amount of the ingested alcohol which is excreted by the urine is seldom more than 3 per cent. Strassman (66) and Völtz and his collaborators (70), (72), (74) usually found that about 1.5 to 2 per cent of 60 cc. of alcohol was eliminated in four or more hours. Spechter (65) did not find such large amounts and Atwater and Benedict (3) in reporting thirteen experiments where human subjects ingested 72.5 grams of absolute alcohol divided into six doses and taken two or three hours apart, found the total elimination through the urine to be scarcely 0.2 per cent. Such a variety of results may be expected since the elimination will be influenced by the amount and dilution of the alcohol taken, the presence of food in the stomach, the amount of muscular exercise following ingestion, and the frequency of urination. If the elimination in our experiments, 1.2 to 1.4 per cent within two hours is larger than that recorded in many others, it should be remembered that we have given a relatively small amount of alcohol in a dilute form and without food, the diuresis was comparatively large (37), there was only a moderate amount of physical exercise, and the urinations were so frequent that there was not so much opportunity for the alcohol to be reabsorbed from the bladder into the blood stream.

DISCUSSION OF RESULTS

1. Relative concentration of alcohol in blood and urine

There are no published data which are strictly comparable with the results presented in this paper. Obviously, alcohol can

be given in such a variety of concentrations and amounts, to say nothing of other conditions, that it is only a matter of chance or purposeful planning if two workers have employed the same dose and about the same routine. For the most part, the experiments on animals have involved relatively large amounts, usually from 2 to 5 cc. per kilogram of body weight. The object has apparently been to introduce enough alcohol to produce an observable effect of some kind. Thus organisms, which are normally abstinent so far as alcohol is concerned, have been practically flooded with it. Probably the majority of experiments on humans have been with doses ranging around 1 cc. per kilogram of body weight taken in wine or more concentrated form, i.e., from 10 per cent to pure alcohol Miles (46), pages 20-23). Only recently has there been any interest in investigating the effects of small amounts given in very dilute form.

Two experiments by Marshall (42) were so conducted as to be fairly comparable with our own in the alcohol concentration for the blood. Marshall had two young men as subjects who, one to one and one-half hours after breakfast, each drank 1200 cc. of 2.75 per cent alcohol by weight, i.e., about 33 grams taken at temperatures from 7 to 10°C. After one and one-half to two and one-half hours following the ingestion, which was accomplished in five minutes, the blood in one case showed 0.28 and in the other 0.26 mgm. alcohol per cc. Considering that the beverage followed the taking of food rather closely whereas in our own experiments, the alcohol was taken on an empty stomach, Marshall's average figure of 0.27 compares pretty closely with the value 0.30 mgm. in the blood (fig. 20). Gies (20) also has published in an abbreviated way a few results using 2.75 per cent alcohol. A subject, forty-eight years old, and weighing 99 kgm., drank between 4:25 and 5:27 p.m. nearly 2200 cc. of 2.75 per cent by weight. One full hour following the completed ingestion, the blood showed 0.86 mgm. per cubic centimeter. A second subject twenty-seven years old; weighing 67 kgm., drank between 4:25 and 5:27 p.m. the same dose and one full hour following ingestion showed 1 mgm. per cubic centimeter of blood. These subjects are said to have eaten a few crackers during the drinking

but supposedly the stomach was otherwise nearly empty of food. Gies' subjects took about 60 grams of alcohol which is more than twice as much as we used. Widmark (78) had an experiment in which a man took 24 grams of alcohol in 1330 cc. of liquid, which equals 1.8 per cent. The subject had been fasting for several hours. One hour after ingestion, the blood showed 0.40 and two and one-half hours after drinking, 0.22 mgm. per cubic centimeter. These are scattered comparisons of blood results obtained after giving dilute alcohol; so far as they apply they appear to substantiate our own results for the amount of alcohol appearing in the blood.

Although the control analyses (fig. 1) show a systematic error between blood and urine samples of about 6 per cent, this is not at all sufficient to account for the difference in alcohol concentration between blood and urine which we have usually found one to two hours following ingestion. Thus our results do not confirm those previously reported. In the paper where Widmark (78) summarizes the more or less chance observations in which different authors have compared single urine samples with blood samples,⁸ he reports a critical experiment which seemed to demonstrate the identity of alcohol concentration for venous blood and urine. His subject, a man weighing 76 kgm., who was used to moderate amounts of alcohol drank 50 cc., i.e., 40 grams of absolute alcohol in a dilute form. At forty-five minutes following ingestion, the subject was catheterized and the catheter kept in place during the rest of the experiment. The urine was collected in three minute intervals. The essential findings of the experiment are as follows:

	MINUTES AFTER INGESTION					
	45	54	60	69	75	81
Mgm. alcohol per cubic centimeter of urine.....	0.98	1.04	1.09	1.00		0.91
Mgm. alcohol per cubic centimeter of blood.....			1.08			0.86

⁸ Other instances of such comparisons are: Gréhant (24), Haneborg (29, page 24), and Maignon (41). See also the papers by Lenoble and Daniel (39), and

Taken as they stand these two blood samples do show very good agreement in alcohol content with the urine samples to which they correspond. They happen to each be a little lower than the urine but that is a minor matter. We are not disposed to question but what such results as these of Widmark *can be found*. Among our own experiments, we have however, no results which closely resemble these of Widmark unless they be the two experiments 10 and 16 where the diuresis was extremely small during the first hour of the experiment. Widmark's blood results, 1.08 and 0.86 mgm. do seem to be extraordinarily high considering the amount of alcohol ingested which was about 0.66 cc. per kilogram of body weight. Schweisheimer (63) gave his twelve subjects 1.57 cc. of absolute alcohol per kilogram of body weight, which is more than twice that used by Widmark and still Widmark found as much in the blood as did Schweisheimer. In the experiment quoted above where Widmark gave 24 grams of alcohol in 1330 cc. liquid and in the blood after one hour found 0.40 mgm. he has a result which is higher than any we have found in our experiments with 27.5 grams given in 1000 cc. The nearest to it is in experiment 19, figure 22, on the pronounced habitual. A further consideration which gives the impression that Widmark's blood results are too high comes from his first paper (76, experiments I and II, pages 11-13). Here he found that the alcohol concentration of the blood was higher than the urine by 13 to 40 per cent.

Assuming identity of alcohol concentration between the blood and urine, Widmark (77) determined the alcohol in the urines of individuals arrested for drunkenness. He reports 27 cases. Three of these show respectively 4.56, 4.16, and 4 mgm. alcohol per cubic centimeter.⁹ Other cases gave 3.44, 3.20, and 3.12 mgm. The average for 23 of those arrested, not all were suffering from intoxication, is 2.32 mgm. alcohol per cubic centimeter

Schumm and Fleishmann (62), for discussions of the alcohol content of cerebrospinal fluid, and Nieloux (50) on the passage of alcohol into the genital secretions.

⁹ Actually as given by Widmark these results are 5.7, 5.2, and 5 parts per thousand which I understand to mean volume per cent. I have converted them into alcohol by weight to conform with the results in this paper.

of urine. In the paper it is emphasized that the presence of alcohol in the urine is the result of a diffusion process and that the proportion can never be higher than that in the blood. Widmark makes the point specific that whatever the content obtained with the urine, one may be confident that the blood then or earlier has at least reached this figure. But his urine results are much higher than any published blood results for profoundly intoxicated individuals. The highest content found by Schweisheimer was in his case (*d*) where probably more than 5 cc. of alcohol per kilogram body weight had been taken and about one hour afterward 1.84 mgm. alcohol per cubic centimeter of blood was found. Schumm and Fleischmann (62) compared the alcohol content of the cerebro-spinal fluid and blood in profoundly drunk individuals. In four cases where the blood sample was secured two hours or less after the last ingestion of alcohol the results were: 0.8, 1.2, 3.04, and 2.16 mgm. per cubic centimeter. They found for their "case 30" 2.08 mgm., and for "case 33" 1.04 mgm. between three to five hours after drinking. In the absence of direct data for comparison it seems that Widmark's high urine results for drunken people therefore give a hint that the alcohol is really higher in the urine than in the blood.

The experiments of Chabanier and Ibarra-Loring (10) performed in Professor Ambard's laboratory are published without much detail and it is impossible to give an adequate critique of them. It is surprising that these and other workers have wholly neglected any direct comparison of blood and urine within the first hour after ingestion and have been willing to assume that these two body fluids are *always identical* in their alcohol content. Apparently the point of view has been somewhat prejudiced by the known readiness with which alcohol diffuses itself through inert membranes, and the fact that the content is more or less parallel in blood and urine whereas most other substances are considerably concentrated for excretion. A similar situation as to point of view seems to obtain in reference to acetone and its appearance in the blood and urine. For example, Widmark (82) in 1920 gave a subject 8 grams of acetone in 200 cc.

of water. Comparable venous blood and urine samples were taken from the man each fifteen minutes for ten periods. "The concentration of acetone in the urine remained throughout somewhat higher than that of the blood. This is probably due to the fact that the acetone in the urine has about the same concentration as that in the plasma which is somewhat higher than that of the total blood." No data for plasma are given in the paper. But at the close of his paper, Widmark makes the summary statement: "Acetone passes into the urine by way of diffusion. The concentration in the blood and in the urine is, therefore, usually the same."

It is very significant that increased diuresis does not alter the concentration of alcohol in the urine. This fact was pointed out by Widmark (78) who made experiments directly on the question, and it has been found in our experiments where at times large fluctuations in diuresis have occurred without intention on our part, e.g., see figures 10 and 11. According to current ideas (Cushny, 11), alcohol as a constituent of the urine would be classed as a "non-threshold" substance since it appears in the urine if there is any at all in the blood. Such substances which disturb the equilibrium of the blood or are not normally present in it are more or less concentrated for excretion. The concentrations in the urine "are invariably reduced in percentage" when there is an increased quantity of urine, and conversely when the amount of urine per minute is small. This is not true of alcohol and in so far indicates a process of diffusion, in which case the blood and urine might be expected to show identity as different workers have contended they do. But our experiments show that although the maxima appear to coincide, the alcohol content in the urine is actually higher than that of the venous blood or plasma. What such a comparison between arterial blood and urine would reveal is not known. Alcohol is oxidized very readily and it is probable, particularly when small amounts have been ingested, that the content in the arterial blood is higher than that of the veins in which case it might more nearly agree with the urine.

2. Alcohol content of corpuscles and plasma

The volume per cent of red cells was determined in most of our blood samples by use of Hedin's Haematokrit. The averages available are from determinations made on 64 blood samples. In relation to the dose given, and at the different intervals following ingestion, the results are as follow:

BEVERAGE	NOR- MAL	MINUTES AFTER DRINKING			
		20	40	70	120
	vol. per cent	vol. per cent	vol. per cent	vol. per cent	vol. per cent
1000-cc. dose.....	42.0	39.3	38.9	39.2	39.9
100-cc. dose.....	42.0	39.9	39.0	39.3	40.0

We had relatively few normal blood samples for comparison with those taken after alcohol but the per cent found, 42, is about average for what is usual for normal blood. The proportion of alcohol that is in the cells as against the concentration in the plasma may be readily calculated. If in a particular instance the whole blood gave 0.36 mgm., the plasma 0.46 mgm. per cubic centimeter and the volume per cent of red cells was found to be 40, then the whole blood value is from plasma and cells in the ratio of 60 to 40. If a sample entirely of plasma gives 0.46 mgm. then $100:0.46::60:0.276$. The whole blood value 0.36 minus 0.276 contributed by the plasma in the sample equals 0.084 which is from the 40 per cent of cells, therefore a sample entirely of cells would show 0.21 mgm. per cubic centimeter. Such a computation on the basis of 40 per cent cells applied to the average results shown in the composite charts, figure 20 and 21, provides us with the tabulated comparisons on page 314. The average concentration in the plasma, 1000-cc. dose, is 0.31 mgm. as compared to an average of 0.17 mgm. for the corpuscles, a concentration ratio of 1.8:1. For the 100-cc. dose the average plasma is 0.47 mgm. and the corpuscles 0.21 mgm., i.e., a ratio of about 2.2:1. According to results obtained by Hedin (32) who mixed cold beef blood with alcohols in 1897, ethyl alcohol distributes itself equally between corpuscles and serum.

	MINUTES AFTER INGESTION			
	20	40	70	120
1000 cc. dose				
	<i>mgm. per cc.</i>	<i>mgm. per cc.</i>	<i>mgm. per cc.</i>	<i>mgm. per cc.</i>
Whole blood.....	0.18	0.24	0.30	0.30
Plasma.....	0.23	0.27	0.37	0.38
Corpuscles.....	0.11	0.20	0.20	0.18
100 cc. dose				
	<i>mgm. per cc.</i>	<i>mgm. per cc.</i>	<i>mgm. per cc.</i>	<i>mgm. per cc.</i>
Whole blood.....	0.28	0.42	0.43	0.36
Plasma.....	0.34	0.53	0.54	0.46
Corpuscles.....	0.19	0.26	0.27	0.21

Data are not now available for a direct comparison of serum and plasma from blood samples following the ingestion of alcohol and it is not clear to what extent, if at all, oxidation of alcohol may take place in the corpuscles when they are in the circulation.

3. Relation of concentration to absorption

In the preceding experiments, with only one exception, *Sut*, nos. 22 and 23, each pair have shown very definitely a higher concentration of alcohol appearing in both blood and urine following ingestion of the more concentrated beverage. The differences shown by the confirmed habitual, *Cef*, were indeed very marked but they are paralleled in the results found by Mellanby (14), (15), who gave 171 cc. of absolute alcohol in various dilutions, to a regular habitual with whom he worked. He found that with the more dilute solution, the content in the blood was lower and reached its maximum at a later time. The difference between habituated and non-habituated individuals which has been made so clear by the researches of Prinsheim and Schweisheimer seems to apply particularly to the more concentrated beverages. While the confirmed habitual seems to absorb the alcohol into the blood the more rapidly, it must be admitted that impairment of liver function is frequently present in such cases and the difference may be on the side of utilization. Both

habituated and non-habituated appear to absorb 2.75 per cent alcohol at about the same rate but they absorb the more concentrated alcohol more readily with a resulting higher concentration in the blood. This problem, as has been recently pointed out by D'Abernon (12), (13), is one of considerable importance from the standpoint of the control of alcohol intoxication. It is true that Widmark (78) gave to one subject without food about 22 cc. of absolute alcohol diluted on different days from 3.5 to about 35 per cent by weight and that he shows *urine results* which indicate that "the dilution of the alcohol has no noticeable influence upon the concentration of alcohol in the blood or the urine." This result has been found on one subject and it is entirely contrary to all of the results which have been obtained by Mellanby in working with dogs and human subjects as well as to the results presented here. In our own experiments, it has been pointed out that while the concentrated dose produced a maximum in the blood that was 50 per cent higher than that from the dilute dose, the difference between the maximum for the urine samples was more nearly 30 per cent. Widmark's results in reference to this point resemble those which we found in experiments on *Sut* (figs. 24 and 25), which we must conclude are not typical for the influence of concentration upon absorption.

SUMMARY

This research grew out of the question whether or no the alcohol content in the urine is identical with that in the blood and if these parallel the intensity of intoxication or alcohol effect following the ingestion of one portion. The Widmark-Nieloux method of alcohol determination has been employed and men representing abstinent, moderate drinkers, and habituels have been tested.

1. The alcohol concentration in venous blood and in urine after taking 27.5 grams absolute ethyl alcohol diluted in 1000 cc. or 100 cc. of fluid is not identical and does not run parallel in the first two hours after ingestion. Taken as 2.75 per cent, the concentrations in the venous blood at 20, 40, 70, and 120 minutes after ingestion are 18, 24, 30, and 30 mgm. of alcohol per

100 cc. The corresponding urine values are 15, 37, 47, and 43 mgm. per 100 cc. The urine concentration is 40 to 50 per cent higher than the blood at forty minutes to two hours after ingestion and it is also higher than that of the plasma by 20 to 25 per cent. The alcohol taken as 27.5 per cent solution gave at the above specified intervals, 28, 42, 43, and 36 mgm. per 100 cc. in the blood, and in the urine 24, 51, 65 and 49 mgm. per 100 cc.

2. The same weight of alcohol taken in a concentrated solution produces a definitely higher alcohol concentration in both blood and urine than when taken in a much more dilute solution.

3. During the first twenty or thirty minutes after ingestion the urine alcohol is about the same or a little higher than in the blood; then for one hour or more it is 35 to 50 per cent higher.

4. The plasma contains a higher alcohol content than the corpuscles in the ratio of about 2:1, but the urine is usually higher than the plasma.

5. Subjects thoroughly habituated to alcohol absorb the 2.75 per cent dose about the same as non-habituated individuals.

6. Changes in the amount of urine per minute do not perceptibly influence the concentration of alcohol in the urine.

7. The per cent of alcohol eliminated in the urine within two hours after ingestion is 1.2 to 1.6 of that ingested. The major part of the elimination occurs in this period provided the bladder is emptied two or three times.

8. Both blood and urine reach their maximum alcohol concentration at about the same time and while not identical still the urine-alcohol curve is very useful for comparison with the time relations of the objective measurements of the alcohol effect on the central nervous system.

For assistance and criticism of this work, I am under deep obligation to my colleague, Dr. T. M. Carpenter. It was in his biochemical laboratory and under his supervision that many of the early analyses were carried out. Dr. H. F. Root has most kindly coöperated in taking all of the blood samples and I have profited by discussion of the problem with Dr. H. Berg-

lund, who first suggested to me the comparison between plasma and corpuscles. To Dr. J. C. Aub who assisted in securing the subjects and to the gentlemen who served in that capacity, I would express gratitude.

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THE EFFECT OF EPINEPHRINE ON EXCISED STRIPS OF FROGS' DIGESTIVE TRACTS

CHARLES M. GRUBER

*From the Department of Pharmacology, Washington University School
of Medicine, St. Louis*

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In studying the effect of epinephrine on the smooth muscle of the esophagus and stomach in cold blooded animals a variety of methods has been used by numerous observers with varying results. Bottazzi and Torretta (1) and Bottazzi (2) used solutions of adrenalin¹ ranging in strength from 1:1000 to 1:150,000. They found that solutions of 1:20,000 to 1:50,000 produced relaxation of the longitudinal coat of the toad's esophagus (*Bufo vulgaris*) but that a solution of 1:120,000 had no effect. Bottazzi also noted that if the adrenalin solution was not too strong nor applied too often this relaxation was in some cases followed by increased tonus.

A similar loss in tonus was noted by Boruttau (3) when "rings" of a frog's esophagus were moistened with adrenalin in solution. In 1899, he also observed a rapid loss of tonus in the excised ring of the frog's stomach when it was painted with a solution of suprarenalin. Three years later, Dixon (4) found that suprarenal extract increased the tonus and exaggerated the waves in the intact stomach of the frog when injected into the circulation and if it was applied to a stomach paralyzed by cocain or nicotin relaxation resulted. His observations were supported, in part, by Meyer (5) in 1906, who reported that a 1:10,000,000 adrenalin solution caused a rapid increase in the rhythmical contractions but that 1:1,000,000 and 2:1,000,000 solutions caused a cessa-

¹ In this review the author will adhere to the nomenclature used in the original articles.

tion of contractions for an hour and in many cases permanently in the stomach muscle.

Excised rings of the stomach of *Rana esculanta*, with and without the mucous membrane, were used by Kautzsch (6) in 1907. In his experiments suprarenalin caused relaxation. However, in one instance in a ring of mucous membrane, it induced rhythmic contractions which had not been present and increased to a marked degree the tissue's sensitivity to thermal stimuli. A control ring remained quiescent.

Hopf (7) in 1910, using the same method as that used by Dixon, remarked inhibition of the frog's stomach when adrenalin (1 per cent solution) was applied to it with a brush.

I know of no one who has attempted the determination of the action of epinephrine upon the excised intestinal muscle, ileocolic sphincter, rectum and cloaca of the frog. This work was undertaken to determine, if possible, the cause of the differences in results of other observers on the stomach of the frog and also to determine the action of adrenalin on the other gastro-intestinal organs of the same animal.

METHOD

Common leopard (*Rana pipiens*) and bull frogs (*Rana catesbiana*) were used in these experiments. Some of the animals were recently caught, others had been kept in the aquarium for periods of one to six months. After pithing the animals, the entire alimentary canal was excised and placed upon a clean glass plate previously moistened with Ringer's solution. Longitudinal and circular strips of the desired parts of organs were prepared, some with and some without the mucous membranes, and attached as quickly as possible to the recording apparatus where they were bathed in Ringer's fluid through which oxygen or air was kept constantly bubbling. A bent glass tube with a T-shaped end was mounted on an iron stand at such a height that a 100 mil. beaker could be slipped up over the T. A wooden stand was then slipped beneath the beaker for its support. Air or oxygen which bubbled through tiny openings in the T-tube was conveyed to the solution through the glass tube. The

strips were suspended in a vertical position, the lower ends fastened to small metal hooks on the T-tube and the upper ends connected by means of serrefines and linen thread to the short arms of the writing levers. The four light muscle levers (heart levers) were arranged one above the other so that their points formed points on a vertical line on the drum surface when the writing arms of the levers were in the horizontal position. These magnified the contractions six times. The amount of tension applied to each tissue was usually just less than sufficient to counterbalance the weight of the tissues and serrefines when the tissue was out of the solution.

An electro-magnetic signal, which was connected by dry cells to a chronometer marked five-second intervals and indicated the point at which the solutions were changed.

Adrenalin chloride and epinephrine (Parke, Davis preparations) were used in all experiments. No difference was observed in the effects of the two solutions and they will therefore both be called adrenalin hereafter. As soon as the contractions and tonus became fairly regular the adrenalin baths were prepared. In each of five beakers was put 100 mls. of Ringer's solution. Enough Ringer's was removed and adrenalin added to make dilutions of 1:1,000,000,000, 1:100,000,000, 1:10,000,000, 1:1,000,000 and a 1:100,000. In a few cases a 1:10,000 and 1:1000 solution was used. It is important to note that the solutions were always applied in increasing strengths. In every case after the adrenalin bath was removed from around the strips any remaining fluid containing adrenalin was rinsed off and fresh Ringer's substituted. In this way the tissues were kept in as nearly constant a solution as possible. The solutions were of room temperature, about 22° to 26°C.

RESULTS

Esophagus. My results upon the esophagus are not entirely in accord with those found by Bottazzi and Torretta (1) and Boruttau (3) who found that adrenalin caused relaxation of the organ.

Longitudinal muscle. In some cases the entire esophagus was used, in others I used a strip 3 mm. wide by 2 cm. in length. Adrenalin in weak solutions produced increased tonus in only a few of these strips. The characteristic action is inhibition (see tables 1 and 2 and figure 1).

Figure 1 is presented to illustrate the action of adrenalin upon the longitudinal muscle of three of the organs of the frog's alimentary canal. The upper curve is a record of the contractions of the isolated esophagus.

It will be seen from the figure that adrenalin at (2) and (3), 1:10,000,000 and 1:1,000,000 dilutions respectively, had no apparent action either upon tonus or rate of contraction. At (4) the muscle was bathed in a 1:100,000 solution for ninety seconds and a relaxation of tonus and cessation of the contractions resulted. The strength of solution of adrenalin which will inhibit contractions of the longitudinal muscle of the esophagus is not constant for the different animals but varies with each (see table 1).

Circular muscle. Rings which measured 3 mm. wide were cut from different parts of the esophagus; split and suspended as a strip of tissue. In most experiments a segment was taken just above the cardiac sphincter, but in many cases two strips of esophagus were used one from near the pharynx the other from near the cardia.

The results with weak solutions do not support the conclusions of Boruttau (3) but the results of strong solutions do. In many cases in which the contractions were feeble a weak solution of adrenalin markedly increased their force and rate. This increase can best be seen in figures 2, 3, and 4, and in table 1. In figure 2 at (3) the tissues were subjected to a 1:10,000,000 solution of adrenalin for 130 seconds. The force of the contraction of the esophagus was increased by more than 113 per cent. A similar change is observed in figure 3 at (1) in which the muscle was exposed to a solution of similar concentration with a resultant increase in the extent of the contractions and a more regular rhythm. Up to a certain point as the strength of the solution is increased the tonus of the esophagus is increased or there is a

TABLE I
The effect of increasing concentration of adrenalin chloride upon excised strips of the frog's alimentary canal. Table showing the number of experiments performed and the results of each

MUSCLE OF ORGAN	ADRENALIN SOLUTION															
	1:1,000,000,000		1:100,000,000		1:10,000,000		1:1,000,000		1:100,000		1:10,000		1:1,000		1:100	
	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0
Longitudinal esophagus.....		6			5	12	1	16								
Circular esophagus...		6			8	15	14	2								
Cardiac sphincter....	1	1	1	3	2	1	2	3		1						
Pyloric sphincter....	1	1	1	3	3	2	3	4								
Stomach*.....		4	5	7	8	11	6	14	1							
Longitudinal small intestine.....	2	2	11	6	26	3	14	5								
Rectum*.....	1	5	4	6	12	7	9	15								
Cloaca*.....	2	3	6	5	12	6	9	16								

* Circular and longitudinal muscle included.

+ Increased tonus or increased force of contractions or both.

+ - Increased tonus followed by relaxation.

+ - + Increased tonus followed by loss with subsequent increased tonus.

- - + Relaxation followed by increased tonus.

- Loss of tonus or cessation of contractions or both.

0 No apparent change.

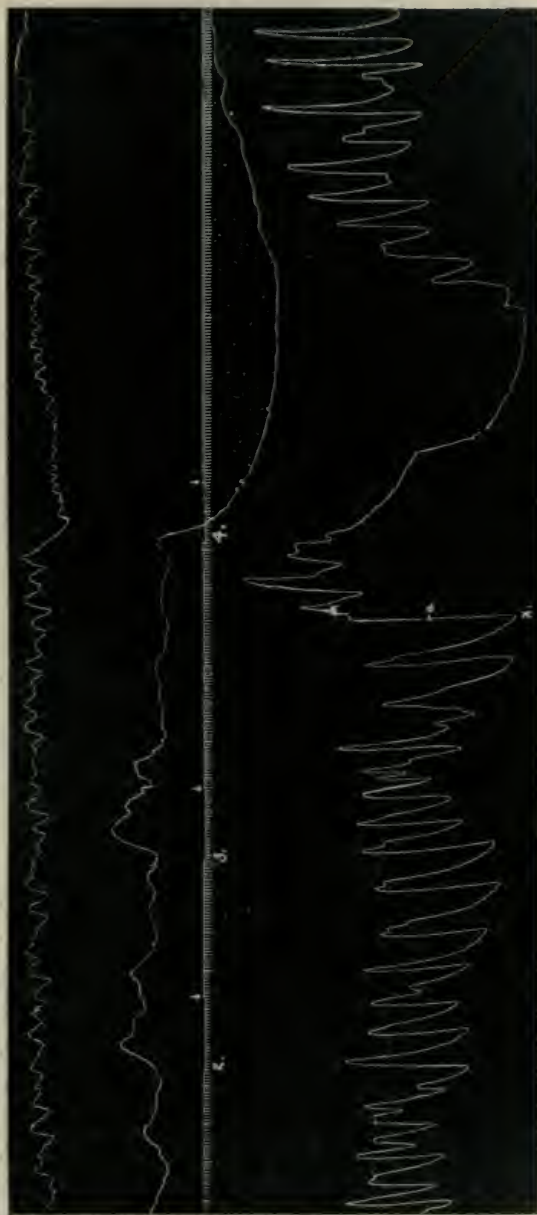


FIG. 1. *RANA PAPIENS*

Upper curve, longitudinal muscle of the esophagus; middle, longitudinal muscle of the rectum; lower, longitudinal muscle of the cloaca. Adrenalin chloride (2) 1:10,000,000; (3) 1:1,000,000; (4) 1:100,000. In this and all following records the adrenalin bath extended only from numeral to the arrow on the time line. Time interval in all is five seconds. All records read from left to right. Curve reduced $\frac{2}{3}$.

fall in tonus followed by a marked increase or a temporary rise in tonus followed by a fall, then by a prolonged rise. In a few cases only a fall was observed, but this was rare. A much stronger solution was necessary to produce the maximum increase in tonus of the ring from the upper esophagus than with the ring taken from the lower esophagus near the cardiac sphincter. Likewise a much stronger solution was necessary to cause relaxation of tonus in the upper esophagus than that of the lower esophagus. This can be seen in tables 1 and 2 and figures 3 and 5.

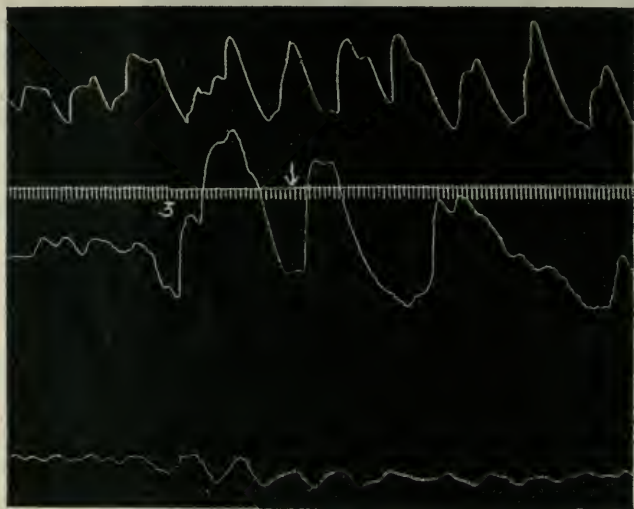


FIG. 2. RANA PIFIENS

Upper curve, circular muscle of the esophagus; middle, stomach; and the lower, the rectum. (3) Strips bathed in adrenalin chloride, 1:10,000,000.

In figure 3 can be seen the effect upon the circular muscle of the esophagus of increasing the strength of the adrenalin solution. At (1), the tissue was subjected to a 1:100,000,000 solution for 175 seconds; a slight increase in tonus and a marked increase in the force of the contraction are seen. At (2) a 1:1,000,000 solution was used for 170 seconds and at (3) a 1:100,000 adrenalin solution for 145 seconds. A 1:10,000 solution produced relaxation of the ring. In figure 4, the same increase in tonus is observed with a 1:1,000,000 solution when the esophagus was

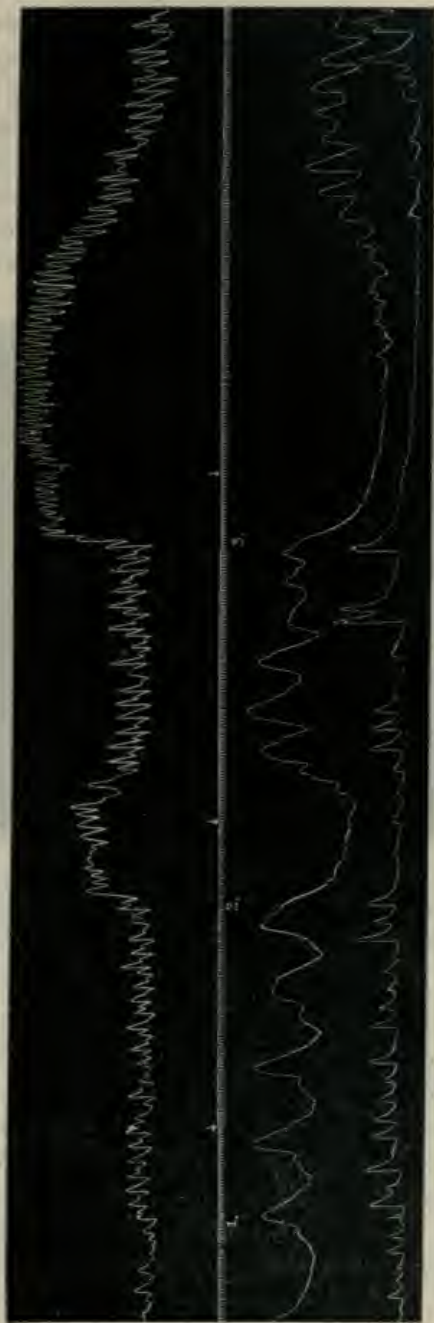


FIG. 3. *RANA PIPENS*

Upper curve, circular esophagus; middle, longitudinal muscle of the stomach; and lower, circular muscle of the stomach. Adrenalin chloride (1) 1:10,000,000; (2) 1:1,000,000; (3) 1:100,000. Reduced $\frac{1}{2}$.

mmersed for 315 seconds. In some cases, after adrenalin in 1:100,000 or 1:10,000 solution was applied to the tissue, a prolonged increase in tonus was observed. In one case this exaggerated tonus lasted for one hour and fifteen minutes, after being bathed in a 1:10,000 solution. In another instance it lasted over one hour. Figure 5 is one such curve in which the tonus lasted for about twenty-eight minutes after the tissues were placed in a 1:10,000 solution of adrenalin for two minutes. Two strips of

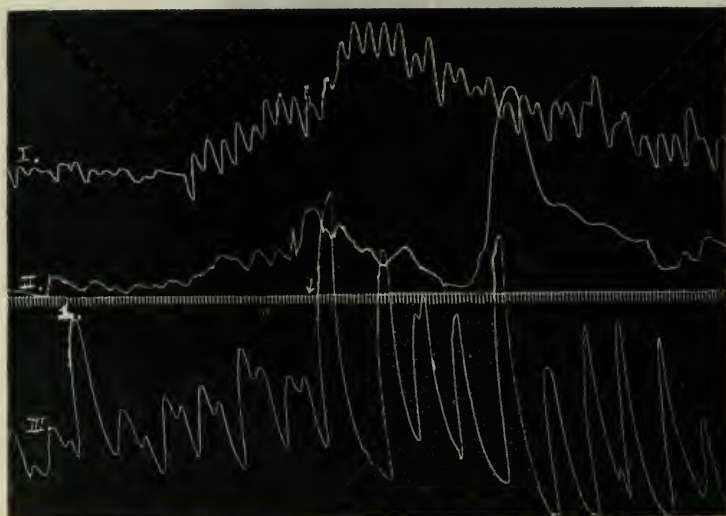


FIG. 4. *RANA CATESBIANA*

Upper curve, circular muscle of the esophagus; middle and lower curves the cardiac and pyloric sphincters respectively. (1) Adrenalin chloride 1:1,000,000. Frog starved six months. Reduced $\frac{1}{2}$.

the circular muscle of the esophagus were used, the upper one taken from near the pharynx, the lower one from near the cardiac sphincter of the stomach. As can be seen in spite of the rinsing of the strip and replacing the bath by fresh solutions at (x) the tonus remained high. Figure 6 (upper curve) illustrates well the usual effect of very strong solutions of adrenalin upon the circular muscle strip. In this there was a loss of tonus which persisted for one and one-half minutes, during which the muscle was



FIG. 5. RANA PAPIENS

Curves of upper and lower esophageal "rings" respectively. (1) Epinephrine 1:10,000. (x) Fresh Ringer's applied. Reduced $\frac{1}{2}$.

bathed in a 1:100,000 solution of adrenalin. This was followed as usual by a marked increase in tonus.

Stomach. It is interesting to note that many observers believe the first requisite to good contraction was that the frog be recently caught or fed a few days before the experiment. There are some who take the opposite stand. Glassner (8) believed starved animals gave decidedly better results than fed animals. Schultz (9) says he found spring animals quite unsuitable for experimental purposes on plain muscle. Dixon (4) found starved

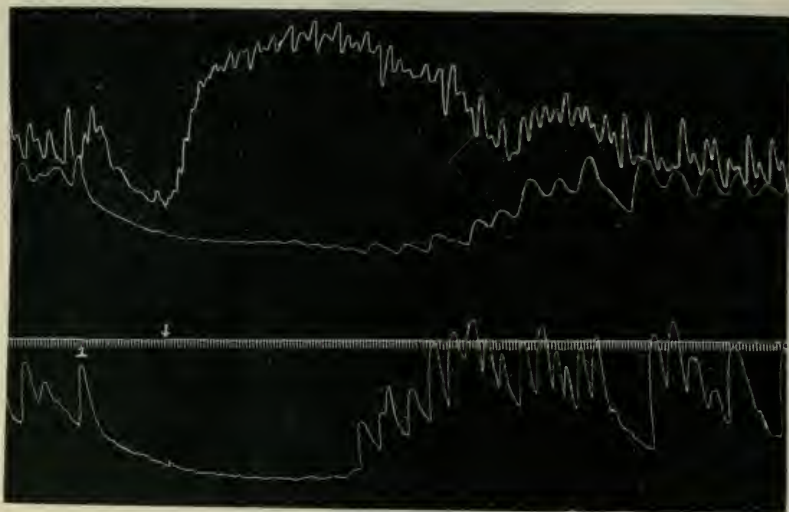


FIG. 6. RANA PIPIENS

Upper curve, circular muscle of esophagus, middle and lower curves, the cardiac and pyloric sphincters respectively. (1) Adrenalin chloride 1:100,000. Reduced $\frac{1}{3}$.

animals distinctly inferior to normal animals and he goes so far as to say that frogs kept in captivity for two months are useless as experimental animals. Morishima and Fujitani (10) found that fed animals gave better results than other frogs kept in captivity unfed. Hopf (7) like Dixon and others found that better results were obtained by the use of recently caught frogs. He also found that "winter frogs" were good if fed a day or so before being used as the stomach contractions in these animals were more powerful than in those of unfed animals.

My experiments were performed upon recently captured frogs and frogs that had been kept in captivity from two weeks to two months. Three medium sized bull frogs (*Rana catesbiana*) that had accidentally been left in an aquarium the previous spring were also used. These animals had thus been kept in captivity for more than six months and the only food possible for them to have obtained was the occasional fly or other insect that may have fallen into the aquarium. These animals were suffering from extreme inanition as can be seen in table 3 and comparing the weights of these animals with those of normal animals of the same species with the same body measurements. The animals remained inactive and when disturbed the movements were sluggish. Upon pithing the animals there was no oozing of blood from the puncture wound. When the abdomen was opened there was little or no hemorrhage from the cut vessels and when the ventricles were excised the blood was pink and watery. The contracting ventricles did not show the usual flushing alternating with paling observed in the contracting ventricle of a normal animal. The stomachs and intestines were pale, small and cord like. They contained no food but some mucous. The recti were empty. The livers were small, dark brown to black in color, very tough and flabby. The gall bladders were opaque, large and greenish black. They contained a thick tenacious, tar-like bile in which was mixed small homogeneous masses. The muscles while intact were pale and atrophic. Before the animals were weighed they were wiped as dry as possible. The organs were placed on filter paper before weighing to remove the excess of body fluids and the lungs were stripped as free from blood as possible.

Upon studying table 3 it will be seen that the animals weighed about half the normal for frogs with skeletons of similar size. The fat bodies were most affected, their loss being 100 per cent, the muscles and liver 70 per cent each. Of the other organs the spleen suffered 83 per cent loss, but the fact that the normal frogs had a large volume of blood and the starved were anhydremic must be taken into consideration. These findings agree closely with those found by Voit (11) on a starved cat.

TABLE 3
Comparative weights of the organs of starved and normal frogs having the same measurements. The starved animals are numbered

	NO. 1*	NO. 2	NORMAL	NORMAL AVERAGE	PERCENT LOST	NO. 3†	NORMAL	PERCENT LOST
Breadth of upper jaw.....	44.0 mm.	52.0	52.0	52.0		49.0	50.0	
Tip of snout to tip of urostyle...	120.0 mm.	140.0	138.0	140.0		128.0	129.0	
Tip of snout to tip of hallux.	300.0 mm.	340.0	339.0	341.0		320.0	325.0	
Weight in grams.	77.525	172.0	304.5	332.0	46.0	115.5	280.7	59.0
Alimentary canal.....	2.290	8.63	11.17	11.14	22.5	5.158	9.89	48.0
Liver.....	0.730	2.693	5.60	8.26	61.0	1.398	6.65	79.0
Gall bladder.....	1.230	1.822	0.62	0.57	Gain	1.330	0.48	Gain
Spleen.....	0.035	0.083	0.20	0.64	80.3	0.042	0.298	86.0
Ventricle.....	0.120	0.495	0.46	0.63	10.0	0.270	0.440	38.5
Kidneys.....	0.205	0.73	1.01	0.89	22.0	0.350	1.095	68.0
Testes.....	0.035		0.38	0.45				
Female reproductive.		2.720				2.120	7.480	71.6
Gastrocnemius.....	0.700	2.010	6.04	7.02	69.2	1.420	5.60	69.6
Lungs.....	0.388	0.822	1.16	1.44	36.8	0.590	1.42	58.5
Fat bodies.....	0	0	2.42	2.78	100.0	0	2.65	100.0
Skeleton, muscles, etc.....	71.792	151.247	275.44	298.18	47.3	102.922	244.609	58.0

* No comparison made as all the normal frogs were much larger than this animal.

† Normal frog was slightly larger than starved animal.

The curves shown in figures 4 and 7 to 10, inclusive, were made by strips taken from the organs of starved frog (2) and are offered as evidence of their activity. In this animal the strips, the re-

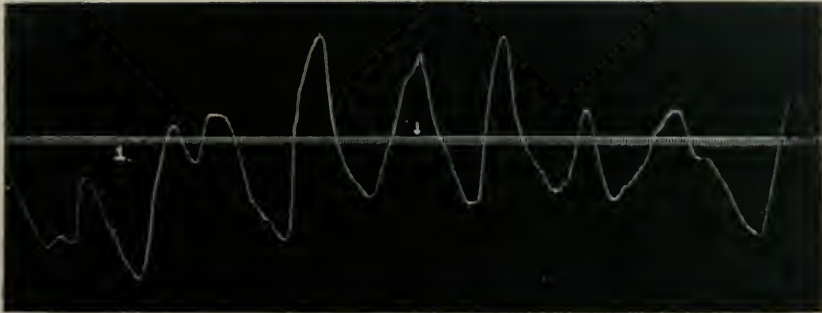


FIG. 7. *RANA CATESBIANA*

Longitudinal muscle of duodenum. The muscle later returned to the base line as seen in the following figure. (1) Adrenalin chloride 1:1,000,000,000. Frog starved six months. Reduced $\frac{1}{2}$.

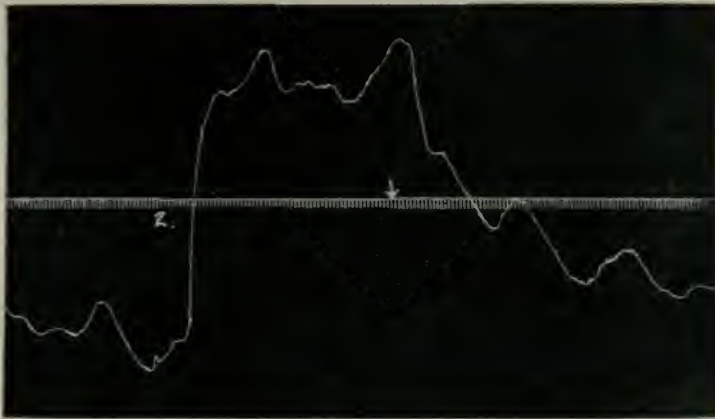


FIG. 8. SAME AS FIGURE 7

(2) Adrenalin chloride 1:1,000,000. Reduced $\frac{1}{4}$.

sults of which are recorded in figure 4, contracted vigorously for more than three hours. These were later replaced by the strips which wrote the curves in figures 7 to 10 which were still contracting after a period of four hours. The strips taken from the



FIG. 9. SAME AS FIGURE 7

(I) Longitudinal strip of intestine; (II) ileo-colic sphincter. (1) Adrenalin chloride 1:100,000. Reduced $\frac{1}{3}$.

different parts of the alimentary canal of the other two starved animals contracted forcefully and rhythmically as soon as they were connected with the recording apparatus and bathed in oxygenated Ringer's fluid.

Longitudinal muscle coat. According to Ranvier (12) the muscular coat of the frog's stomach consists entirely of circular fibres which are the active agents in producing contraction and not the muscularis mucosa. This conclusion is not supported by the observations of Morgan (13), Winkler (14), Schultz (9) and

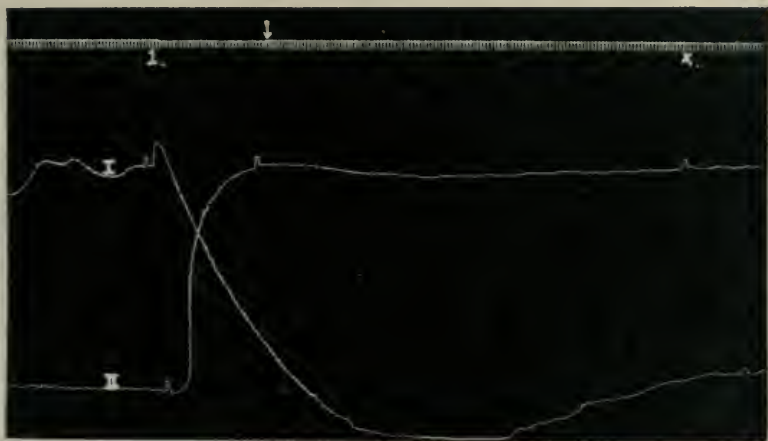


FIG. 10. SAME AS FIGURE 9

(1) Adrenalin chloride 1:10,000. Reduced $\frac{1}{3}$.

Grützner (15), who discuss at length the question of longitudinal fibres.

The longitudinal muscle strip of the stomach, 4 mm. by 2 cm. was taken from different parts of the organ but usually from the pyloric half. It was mounted in the usual manner. In figure 3, the middle curve is a record of such a strip. As can be seen from the curve after the strip was placed in a 1:10,000,000 solution of adrenalin at (1) for 175 seconds the contractions became almost twice as rapid and somewhat stronger than before. These decreased again when the tissue was bathed in normal Ringer's. A 1:1,000,000 and a 1:100,000 solution for 170 and 145 seconds

respectively caused marked relaxation of the tonus and a disappearance of the contraction. In some cases where the contractions were not present or were feeble, adrenalin stimulated them into activity. This observation was made upon other strips as well. The production of spontaneous contractions by adrenalin is in accord with the findings of Mira and Fontes (16). They found that the spontaneous contractions of the excised duodenum of rabbits from which the suprarenal capsules were removed were small or absent. However, they found that animals in which the suprarenal bodies were removed would present the usual contractions if the animals were previously treated with suprarenal extracts. They therefore attributed the spontaneous contractions of the intestinal strips to the action of the cortex of the suprarenal bodies.

Circular muscle coat. The circular muscle coat like the longitudinal muscle was stimulated in some cases to show rhythmical contractions which had not been present before, and in other cases where they were present, they were increased in force when exposed to a weak solution of adrenalin. The middle curve in figure 3 and the lower curve in figure 4 are records of the circular muscle of the stomach taken near the pyloric sphincter. In figure 3 at (3) it will be seen that the circular muscle increases in tonus and in force of the contractions when surrounded by a 1:10,000,000 solution of adrenalin chloride for 130 seconds. After removal of the solution it returned again to its original length after about four minutes. A similar increase is observed in figure 4 at (1). At (2) a 1:1,000,000 solution produced inhibition and at (3) a 1:100,000 produced the same result.

The small intestine. In most cases only the duodenum was studied but in some (six experiments on as many animals) the longitudinal strips from the duodenum, jejunum and upper and lower ileum were studied simultaneously. All the strips were about 2 cm. long. The response of the duodenum to adrenalin was very much the same as that of the stomach. Small doses cause increased tonus and force of contraction and large doses the reverse. Figure 7 is a curve which shows the action very well. At (1) the Ringer's was replaced for over three minutes by

a solution of adrenalin (1:1,000,000,000). The contractions increased in force and the tonus was also increased. The muscle relaxed to its normal length before figure 8 was taken. In this figure a 1:1,000,000 adrenalin solution was used and the muscle increased in tonus at once, remaining thus until the Ringer's was substituted for the adrenalin. Very strong solutions have the opposite action, that of producing lengthening of the muscle and inhibition of the contractions. Figures 9 and 10 (curves I) are records of the same strip of intestine in which 1:100,000 and 1:10,000, respectively, of adrenalin were used. Marked relaxation resulted in each case. This relaxation becomes very quickly maximum as a rule and the muscle remains in this state for some minutes, after being replaced in the Ringer's.

Of the four intestinal strips recorded in figure 11, the duodenum upper record does not respond as readily to solutions of adrenalin (1:10,000,000 and 1:1,000,000) at (1) and (2), respectively, as the others—jejunum, upper and lower ileum. However, there seems to be greater excursion of contraction of the duodenum due to relaxation permitting greater contractions, though the actual shortening of the muscle was not increased. At (3) a 1:100,000 solution of adrenalin caused relaxation in all the muscles. In some other experiments this difference between the duodenum and ileum is not so marked.

Cut circular muscle strips of the small intestine responded to adrenalin in the same manner as did longitudinal muscle strips.

Rectum and cloaca. In as much as it is impossible to determine the dividing line between the rectum and cloaca these two organs will be discussed together. The rectum according to Parker and Haswell (17) passes without change in diameter into the cloaca. The cloaca would therefore be the lower end of the rectum. It has been said also that the part of the large intestine containing feces is the rectum and the lower part containing no feces the cloaca. This was the differentiation used in preparing muscle strips from these parts. In all cases the bladder was stripped from the cloaca before it was excised.

Both longitudinal and circular strips responded to adrenalin in the same manner. A study of the tables will show that the

rectum and cloaca were equally responsive to stimulation by adrenalin and that about the same concentration of the solution caused relaxation in the two tissues.

The contractions were inhibited more readily in the rectum, those of the cloaca in the majority of cases continuing even though the tonus fell to a marked degree.

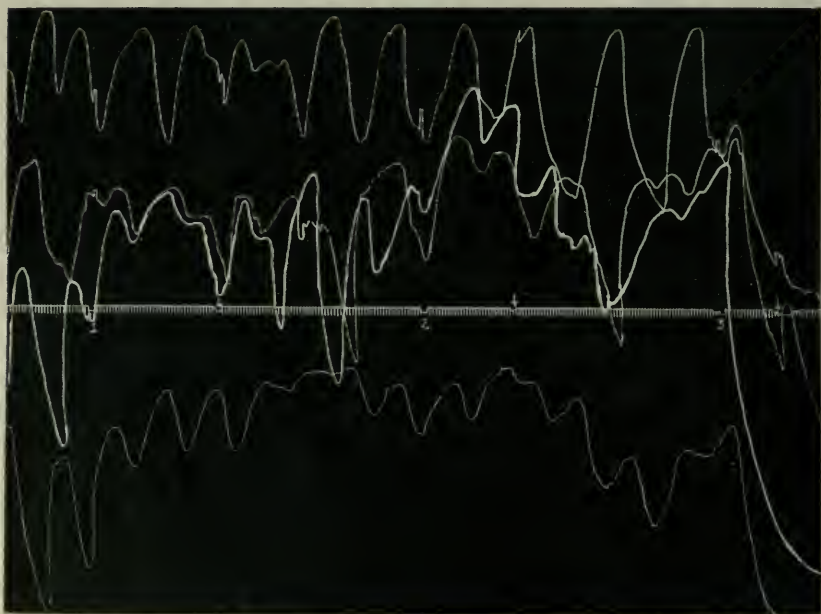


FIG. 11. *RANA CATESBIANA*

Upper curve, upper duodenum; second from top, lower duodenum or jejunum; heavy curve, upper ileum; lower curve, lower ileum. Adrenalin chloride (1) 1:10,000,000; (2) 1:1,000,000; (3) 1:100,000. Reduced $\frac{1}{2}$.

In figure 1 can be seen the effect of adrenalin upon the longitudinal muscle coat of these two organs. The middle curve is the rectum, the lowest curve the cloaca. Adrenalin (1:10,000,000) caused increased tonus in the rectum but no apparent change in the cloaca except a possible increase in the force of the contractions. At (3) the solution (1:1,000,000) caused increased tonus of both rectum and cloaca while at (4) 1:100,000 produced a marked relaxation of both strips. In practically all cases the

cloaca recovered from the effects of the adrenalin more quickly than the rectum. The increase of tonus in the rectum occurred almost simultaneously with the relaxation of the muscle of the esophagus after the removal of the adrenalin solution. See figure 3. In figure 2 the lowest curve is that of the rectum. At (3) can be seen the increased force of contraction as a result of adrenalin (1:10,000,000).

Cardiac and pyloric sphincters. In the cardiac and pyloric sphincters as in the stomach weak solutions of adrenalin cause contraction and strong solutions produce relaxation. In figure 4 adrenalin (1:1,000,000) produced increased tonus in the cardiac

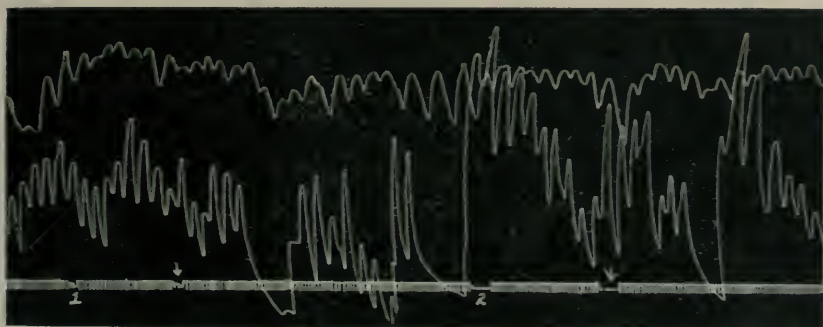


FIG. 12. RANA CATESBIANA

Upper curve, cardiac sphincter; lower, pyloric sphincter. Epinephrine (1) 1:1,000,000,000; (2) 1:100,000,000. Reduced $\frac{1}{2}$.

sphincter and a gradual increase in the force of the contractions in the pyloric sphincter. In the latter when the adrenalin was replaced by Ringer's a long series of strong contractions followed. In figure 12 at (1) a 1:1,000,000,000 solution of adrenalin was used without effect but at (2) a 1:100,000,000 solution increased the tonus especially in the pyloric sphincter. The effect of a 1:100,000 solution of adrenalin is noted in figure 6. Both sphincters relaxed as a result of the substitution of the adrenalin for the Ringer's for 135 seconds.

Ileo-colic sphincter. In some cases the ileo-colic sphincter reacted in the same way as the lower ileum, a weak solution of adrenalin causing contraction, a stronger solution causing re-

laxation. However, this sphincter responded almost invariably with contraction to all strengths up to 1:100,000. In figure 9, curve II at (1), the sphincter and intestine were bathed in a 1:100,000 solution of adrenalin. The sphincter responded within one minute by a powerful contraction. This increased tonus was maintained for some minutes. In figure 10, curve II, a 1:10,000 solution was used. The latent period was much shorter with this stronger solution and the shortening more complete. This shortened state was maintained for almost one hour after the adrenalin was rinsed off and replaced by Ringer's. The intestine in each case relaxed.

Comparative action of adrenalin upon the different parts of the alimentary canal. Upon studying the curves collectively and the results of all the experiments collected in table 1 it will be seen that a strength of solution sufficient to cause contraction in the intestine is inadequate to effect the esophagus. In all the organs the weak solution produced contraction and the stronger produced relaxation.

In figure 1, the contractions of strips of longitudinal muscle of the esophagus, rectum and cloaca were recorded simultaneously. Adrenalin (1:10,000,000 and 1:1,000,000 solutions) at (2) and (3) had no action upon the esophagus but produced contraction in the rectum and cloaca. Adrenalin solution (1:100,000) however, produced relaxation in all three tissues.

A similar illustration is shown in each of figures 3, 4 and 6. In figure 3, strips of the circular muscle of the esophagus, the longitudinal and the circular muscles of the stomach are recorded. At (2) a 1:1,000,000 solution produced contraction of the esophageal strip but relaxation of both stomach strips. This effect is strikingly illustrated in the same figure (3). In figure 2, a solution of 1:10,000,000, just as in figure 4 a solution of 1:1,000,000 adrenalin produced increased force of contraction in all three strips and in figure 4 increased tonus as well. In figure 2, the circular muscles of the esophagus, stomach and rectum were used; in figures 4 and 6, the circular coat of the esophagus and the cardiac and pyloric sphincter strips were employed. Here, however, adrenalin produced a disappearance of the contractions and a

loss of tonus in the pyloric and cardiac sphincters, and a loss of tonus with a decrease in the height of contractions followed by an increase of tonus in the ring of esophagus. There is also a slight increase in tonus in the pyloric sphincter after recovery from the forced relaxation. Still more striking differences can be seen when we compare the curves taken from the ileo-colic sphincter and the duodenum. In figure 9, the duodenum after a temporary contraction relaxed, but the ileo-colic sphincter only contracted. A greater difference can be seen in figure 10 from the same strips of tissue.

It can be seen from these results that it is important to note the strength of adrenalin solution and the part of the canal used in reporting results upon the action of adrenalin on the digestive tract.

An attempt was made in tables 1 and 2 to collect the results of all experiments in groups according to the strengths of adrenalin solution used. The longitudinal and circular esophageal and stomach strips were not irritable to 1:1,000,000,000 adrenalin. The remaining organs were. The percentage of positive results for this dilution in table 2 may be too high because of the limited number of experiments performed. All the tissues except the circular muscle of the esophagus responded to the 1:100,000,000 adrenalin solution. The increased tonus of the longitudinal muscle of the esophagus was not great in any instance. The longitudinal muscle, however, contracted to a less strong solution of adrenalin than was necessary to cause contraction of the circular muscle (see tables 1 and 2).

Some of each variety of strips prepared when bathed in a 1:10,000,000 solution responded by tonic contraction. With this strength, however, some began to show the opposite effect, that of loss of tonus or of cessation of the contractions or both. The chief characteristic response to this strength was still contraction for the majority of tissues though more than half of the strips of the longitudinal muscle of the esophagus relaxed.

With a 1:1,000,000 solution the majority of strips of each variety except the circular muscle of the esophagus and the small intestine reacted with a loss of tonus or cessation of contractions.

When adrenalin was increased to 1:100,000 dilution the circular muscle of the esophagus still showed tonic contraction in 38 per cent of the experiments. All the other tissues relaxed when bathed with this concentration.

In only a few instances was adrenalin 1:10,000 and 1:1000 solution used. Although these concentrations were found to excite some circular strips of the esophagus and ileo-colic sphincter to contraction they nevertheless produced a profound inhibition of tonus and contractions of all other tissues. In all the experiments in which such strong solutions were used the relaxed tissues failed to regain their original strength of tonus and contractions, and in many instances these reactions were completely and permanently lost.

It was important that the solutions be applied in increasing strengths because it was found that in many cases after the tissue was bathed by a solution which caused relaxation, it would no longer respond by contraction or increased tonus to the solution to which it had previously responded. This may be due to the profound action of adrenalin upon the endings of the nerve fibers which bring about the tonic contractions.

It is interesting to note that the rectum is more easily excited to contraction by adrenalin than the esophagus, the intervening tissues decreasingly sensitive as they approach the upper part of the canal.

Tonus waves. Frequently adrenalin caused the tissues to set up violent tonus waves, in some cases to such an extent as to make them unfit for further experimentation.

DISCUSSION

From my results in the frog, I am inclined to attribute to the sympathetic system a double innervation throughout the alimentary canal. Some of the fibres excited by a weak solution of adrenalin cause tonic contraction and in some cases increased force and rate of contraction and other fibres excited as by a strong solution of adrenalin cause loss of tonus in these same organs. The threshold stimulus seems to increase for both responses from the rectum to the esophagus. It does not seem

sufficient to explain the differences in action to adrenalin in the different organs by a difference in metabolism as has been thought to be the case in the rabbit's digestive system by Alvarez and Starkweather (18). It might be argued that the circular muscle of the esophagus contracts in the majority of cases even with strong solutions because the metabolism is so rapid in this organ that the adrenalin solution, although concentrated, can reach the nerve endings only in a more dilute form. However such a simple explanation would fail to account for the fact that various tissues first contracted and then relaxed; that the circular esophagus and the sphincters, cardiac and pyloric, sometimes responded by relaxation followed by an increase in tonus; that some of the circular esophageal muscles responded to adrenalin first by tonic contraction then by relaxation (loss of tonus) and again by tonic contraction. It would also fail to account for the fact that the longitudinal muscular coat of the esophagus responded uniformly by relaxation when a 1:1,000,000 solution of adrenalin was used while the circular muscle failed to respond by relaxation only, with a solution 100 times as concentrated (1:10,000).

The experiments performed in the manner used in my work would, it seems to me, be fair physiological tests of the rate of oxidation of adrenalin in the various tissues. If we assume that the rate of diffusion of adrenalin into or out of the various tissues of the alimentary canal is the same per mass of tissue, then the rate of oxidation of adrenalin must be the same in each. In support of this view figures 3 and 6 are offered.

When the rectum or the stomach and the esophagus are recording simultaneously and the Ringer's replaced by a 1:100,000 or 1:10,000 solution of adrenalin, the two former tissues relax and the latter contracts. In the same way upon the removal of the solution the rectum and stomach strips begin to contract and the esophagus begins to relax almost at the same instant. In each case, I think the tissues are reacting in their individual way to the adrenalin and are not reacting to the solution in opposite ways because of a difference in speed of oxidation.

I believe that there is an actual difference in the sensitivity of the endings of the thoracic-lumbar autonomic nervous system in

the various organs to the adrenalin. If it is reasonable to assume that the nerve endings of these tissues may be stimulated to produce tonic contraction with a weak solution of adrenalin, and relaxation and cessation of contraction of these same organs with a stronger solution it seems conceivable that the sensitivity of these nerve endings may differ from tissue to tissue.

A number of experimenters working upon the frog found a double effect upon the stomach and esophagus when the vagi were stimulated, but no one noted this double action when the thoracic-lumbar autonomic nerves were excited. Goltz (19) 1872 noted that excitation of the vagus caused relaxation of the esophagus and the stomach. Steinach and Weiner (20) reported that excitation of the vagus, of the second and third posterior roots, or of the medulla produced both inhibitory and excitatory actions in the esophagus. They described motor effects upon the frog's stomach, intestine and rectum upon excitation of the vagus and posterior roots from third to seventh, inclusive. In 1897, Horton-Smith (21) failed to confirm Steinach's results and asserted that all efferent fibers pass to the viscera either by the vagus or the anterior roots. In the case of the esophagus the innervation is from the vagus alone. Steinach (22) repeated his experiments and confirmed his former results. Hopf (7) observed contraction and inhibition of the frog's stomach due to vagus excitation. It was noted by Waters (23) that excitation of the third, fourth, fifth and sixth nerve fibres at their point of exit from the spinal cord, produced contraction of the frog's esophagus, stomach and intestine. His findings were confirmed by Contejean (24) who found that excitation of the sympathetic nerve threw the stomach of a toad into a tetanic cramp and by Dixon (4) who found that excitation of the rami communicans of the third, fourth and fifth spinal nerves produced tonic contraction of the stomach but that excitation of the vagus caused relaxation. My results, with the use of adrenalin as a stimulus, do not support Horton-Smith's findings, i.e., that the motor innervation to the esophagus is through the vagi. The sympathetic must send both tonic motor and inhibitory fibres to the esophagus in the frog if we are to explain the action of adrenalin in my experiments. Salvioli

(25) believes that the action of epinephrine is on the muscle directly, but his view is not supported by other observers especially Dixon (26) who found the action of adrenalin to be upon the myoneural junction.

Dixon (4) noted contraction of the frog's stomach when adrenalin was injected intravenously. He did not, however, record relaxation. This was probably due to the fact that he used very weak solutions and intact organs. Langley (27) reported complete relaxation of the rabbit's stomach after adrenalin. Smith (28) experimented upon strips of excised stomach muscle from cats, rabbits, dogs, guinea pigs and humans. He used dilutions varying from 1:10,000,000 to 1:100,000 and observed contraction of the pyloric and cardiac sphincters and relaxation in the strips from the antrum and preantrum. The results upon the body and fundus of the stomach depended upon the animal used; contractions in the rabbit and dog and relaxation in the cat and human. Kuroda (29) found that adrenalin relaxed the cat's stomach. Spadolini (30) maintains that the results of the action of adrenalin upon the dogs' and cats' stomachs are merely a question of the concentration of the solution used, a weak solution 1:80,000 causing relaxation, a stronger solution 1:5000, contraction. His results were not confirmed by Smith (28) who found no difference between weak and strong solutions on the stomach muscles in the animals studied, nor are they confirmed by my results upon the frog. In all my experiments performed (twenty-two in number on as many animals) in not a single instance did adrenalin in 1:100,000 produce anything but marked relaxation and 1:10,000 produced such a profound inhibition and relaxation that in three experiments the organs failed to regain any degree of their original contractions and tonus.

Schiff (31) Morat (32) and Dixon (4) observed the splanchnic nerve to be a motor nerve to the stomach. Wertheimer (33), Elliott (34) and Langley (27) maintained it to be an inhibitory nerve, while Openchowski (35) attributed to the splanchnic, in the dog and rabbit and Spadolini (36) in the dog and cat both inhibitory and motor fibres. Spadolini found that a weak faradic current produces relaxation, but a strong current produced tonic

contraction of the organ. May (37), experimenting with the cat, dog, rabbit and monkey, found no effect upon stimulating the splanchnic with an electric current. He was led to the opinion that the vagus alone supplied motor and inhibitor nerves to the whole of the stomach and to its sphincters and that the splanchnics have no share in the innervation of this organ.

My results upon the frog with adrenalin support Openchowski's and Spadolini's observations that the splanchnic sends both inhibitory and motor fibres to the stomach. If the stimulation with adrenalin is at all analogous to stimulation with a faradic current, we would expect a weak stimulation of the splanchnic to produce contraction and a strong one to produce relaxation. Spadolini reports exactly the opposite.

Though it is generally conceded that the characteristic and predominating action of adrenalin is inhibition of the small intestine (Magnus (37); Langley and Magnus (39); Langley (27); Elliott (34); Dale (40); Cannon and de la Paz (41); Hoskins (42); Alvarez and Starkweather (18); Kuliahko and Alexandrowitsch (43); Kress (44), and many others), we find since early in the history of the study of adrenalin minor reports here and there of its motor action.

In 1897, Ott (45) observed that adrenalin relaxed the intestine and in some cases it increased peristalsis. He made no further mention of the latter fact in his subsequent papers. One year later, Bunch (46) reported, as a result of intravenous injection of 22 mgm. of adrenal extract, an increased tonus of the gut. Magnus (38) working with isolated segments of intestine, saw a single instance in which increased peristalsis occurred following the use of suprarenin but he regarded it as unique.

Salvioli (25) studied the action of the suprarenal extract upon the intestine of rabbits and dogs and noted, when it was injected intravenously, an increased tonus and contraction of the small intestine. He also obtained relaxation of the intestine followed by contraction when the extract was added to the perfusion fluid which passed through the vessels of the excised intestine.

In 1912, Hoskins (42) recorded that in addition to this "characteristic inhibition" by adrenalin there was frequently observed

with dilutions just subminimal to the inhibitory value an increase in the activity of the tissue and often an increase in tonus. In the records cited, he obtained increased activity with a 1:1,000,000,000 and a diminution in activity with a 1:500,000,000 solution in excised rabbit's intestine. Alvarez likewise thought that adrenalin sometimes seemed to increase the rate of contraction of the rabbit's intestine.

Contraction of the muscularis mucosae was observed by Gunn and Underhill (48) with a 1:200,000 solution of adrenalin and epinephrine. According to Kuger and Wijsenbeck (49) in some rabbits' intestines the relaxation, caused by suprarenalin was followed by marked contraction (increased tonus) when the solution was removed. Whether this was due to the diluted solution of suprarenalin or the removal of the suprarenalin they were unable to say.

Spadolini (36) in a series of papers published during the years 1917 and 1918 and a later work, found conditions necessary for relaxation and contraction of the intestine by adrenalin to be opposite those of previous observers. He noted that a weak solution (5 mils. of a 1:200,000) injected intravenously caused tonic relaxation of the segment of intestine in dogs and cats and that a strong solution (3 mils. of a 1:5000) intravenously caused tonic contraction. In another experiment cited by that author, 5 mils. of a 1:200,000 injected in the same manner in the same animals produced inhibition and 3 mils. of a 1:3000 solution produced contraction.

Tashiro (50) without reference to Spadolini's work found that adrenalin in weak solutions caused contraction of the cat's intestine and strong solutions caused relaxation. He separated strips of circular and longitudinal muscle and suspended them in a Ringer's bath and recorded their movements. When 1:100,000 to 1:100,000,000 adrenalin was added to the bath, the circular muscle was observed to increase its tonus and its movements sometimes were augmented. The longitudinal muscle was more sensitive than the circular coat. In a concentration of 1:1,000,000,000 adrenalin the movements were augmented and the tonus frequently increased, but a solution stronger than

1:100,000,000 caused inhibition of the movements and relaxation of the tonus. The results presented here upon the frog's intestine agree with the findings of Tashiro on the longitudinal muscle in the cat and Hoskins on the rabbit, with the same substance. In the frog, 1:100,000 adrenalin solution invariably produced relaxation and in this respect Tashiro's observation on the circular muscle only of the cat differ. The difference found by Spadolini must be due either to the fact that he worked with intact segments of organs or to one condition which he failed to take into consideration, i.e., that 3 mils. of a 1:3000 solution will produce a tremendous and prolonged vaso-constriction in the loops of the intestine and the asphyxia thus produced may be capable of causing the increased tonus observed. Upon analysis of his curves it will be found that in each case the contractions cease entirely and the tonus increases in a curved smooth line.

Statements were made by Ehrmann (51) and Courtade and Guyon (52) that the splanchnic nerve contains, beside the inhibitory fibres augmentative nerve fibres which control the tonus and the movements of the intestines. Bayliss and Starling (53), however, supported by Elliot (34) insisted that in the splanchnic nerve there exist the inhibitory nerve fibres only. Recently Spadolini (36) showed that the splanchnic nerve sends both inhibitory and augmentative fibres to the intestine.

My results with adrenalin on the frog do not support Ehrmann's conclusions that stimulation of the splanchnic caused only the augmentative effect in the longitudinal muscle and the inhibitory effect only in the circular muscle of the small intestine. Nor do they support Courtade and Guyon who state that stimulation of the splanchnic nerve produces the inhibitory effect in the longitudinal only and the augmentative effect in the circular muscle only. I found both inhibitory and augmentative effects in each coat, thus agreeing with two seemingly contradictory sets of results. My results also agree with Tashiro's results with the longitudinal muscle coat of the intestine.

The experiments presented here are, as far as I know, the only ones performed upon the rectum and cloaca in the frog. Langley and Magnus (39) noted that adrenalin causes complete inhibi-

tion of the rabbit's colon and their observations were later corroborated by Alvarez and Starkweather (18). In the dog, Bayliss and Starling (34) observed inhibition of the colon upon stimulation of the splanchnic nerve. Their results were confirmed by Elliott and Barclay-Smith (55). My results with adrenalin agree with theirs but, as is the case in the stomach and intestine, the thoracic-lumbar autonomic nervous system in the frog must also send augmentative and tonic excitative nerve fibres in as much as adrenalin in very minute doses produces contraction of these organs.

To state positively that strips taken from the cardiac and pyloric portions of the stomach and the ileo-colic portion of intestine in the frog are the true sphincters is a rather bold assumption. However, in all cases the portion used was as near the sphincter as could be determined. Langley (56) noted that excitation of the splanchnic produced inhibition of the cardiac sphincter in the rabbit. Cannon (57) found that the cardia of cats offered a temporary obstacle to easy passage of food into the stomach after bilateral vagotomy. His observations are in accord with the findings of many writers but disagree with those of others (58). The splanchnics cause, in the rabbit, contraction of the pyloric sphincter and when adrenalin is given the same result is to be seen according to Elliott. In dogs, splanchnic stimulation is said to have the opposite action, that of opening a closed pylorus (59).

Elliott (60) found that the ileo-colic's tonic closure is due to impulses from the central nervous system by way of the splanchnics. He confirmed his finding by the injection of adrenalin. Kuroda (61) also found adrenalin to cause contraction of the sphincter.

I found that the cardiac and pyloric sphincters responded very much as did the neighboring organs to adrenalin, weak solutions causing contraction and strong solutions relaxation. In some cases the ileo-colic, however, responded to all strengths of adrenalin by contraction.

Is the reversal of action by adrenalin due to a change of the hydrogen-ion concentration of the solution surrounding the tissues?

Snyder and Andrus (62) found that by changing the hydrogen-ion concentration of the perfusate in isolated terrapin hearts, they could modify the reaction of these tissues to epinephrine. If the heart was perfused with a Ringer's solution of pH 7.6 and adrenalin $2.8:10^7$ added, increased tonus and tonus waves resulted if, however, the solution was changed to a pH 7.3 and adrenalin $2:10^7$ added, the muscle showed no increase in tonus or tonus waves but an increase in the amplitude of contraction. Snyder and Campbell (63) demonstrated in 1920 that they could reverse the action of adrenalin upon the frog's blood vessels by changing the hydrogen-ion concentration of the perfusate. They found that adrenalin $1:10^9$ caused vaso-dilatation if the pH was 7.8.

In 1921, Collip (64) published a paper claiming that the hydrogen-ion concentration of the blood was the determining factor as to whether an injection of a small dose of adrenalin would cause the blood pressure to increase or decrease. Although no readings were made as to the hydrogen-ion concentration of the blood, he believed that if it was increased, the same dose of adrenalin caused a fall of blood pressure which produced a rise, if the hydrogen-ion concentration was decreased.

The difference in reaction, to dilute and strong solutions of adrenalin observed in the tissues employed in this research cannot be due to a change in hydrogen-ion concentration of the solution. The original acidity of Ringer's fluid was at all times the same and since the same reactions were obtained with both adrenalin chloride and the alkaloid (epinephrin and adrenalin) the increased acidity by adrenalin chloride can have little added action. This inference can best be seen in figure 13 and in comparing figures 5 and 12 with the other figures presented. In figure 13 (a), between the arrows, the circular esophagus was immersed in a Ringer's solution containing about the same concentration of HCl as a $1:100,000$ adrenalin chloride solution would if the adrenalin were completely ionized. In (b), Ringer's containing adrenalin chloride $1:100,000$ and in (c), adrenalin (alkaloid) $1:100,000$ were substituted, between the arrows, for the original Ringer's. It will be seen that the increased acidity of the solution itself had no effect while the reactions are qualitatively the

same with the use of the salt and alkaloid. The responses of the tissues to adrenalin (alkaloid) dissolved in Ringer's and in Ringer's containing the added HCl as in figure 13 (a) are the same. In figure 14, both curves were written by the same circular strip of esophagus. In the lower curve, between the arrows, the Ringer's fluid was replaced by a 1:100,000 adrenalin (alkaloid) solution. In the upper curve the Ringer's was replaced by a

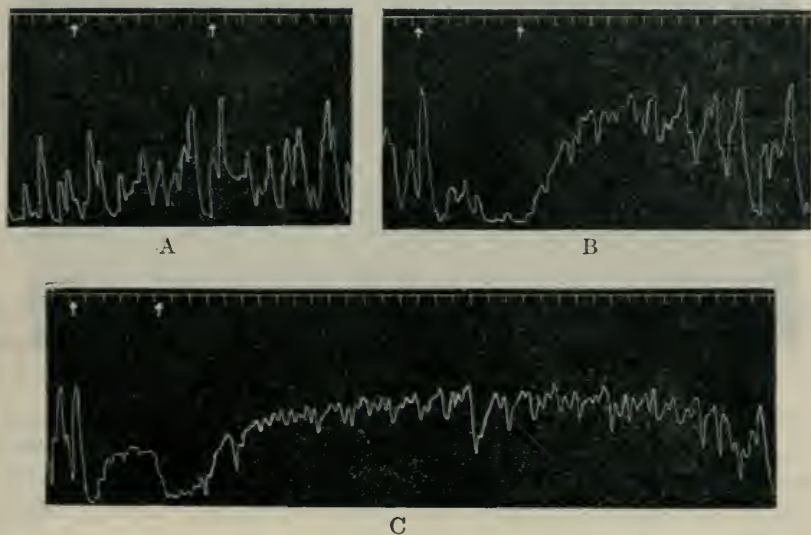


FIG. 13. *RAÑA PIPPIENS*

Circular muscle of esophagus bathed in oxygenated Ringer's fluid. Test solutions applied between the arrows below the time line. Time, thirty seconds. A, HCl 0.000183 grams per 100 mls. Ringer's; B, adrenalin chloride 1:100,000; C, adrenalin (alkaloid) 1:100,000. Reduced $\frac{1}{2}$.

1:100,000 adrenalin (alkaloid) solution in which HCl was added to increase the acidity as in figure 13 (a). The curves are essentially the same irrespective of the change in acidity of the solution. We are, therefore, forced to conclude that the change in acidity of the Ringer's solution by the addition of adrenalin chloride had no effect upon the results obtained with weak and strong solutions of adrenalin.



FIG. 14. RANA PAPIENS

Circular muscle of esophagus. Upper curve, adrenalin (alkaloid) 1:100,000 in Ringer's to which was added HCl 0.000183 grams per 100 mls. was substituted for the Ringer's solution between the arrows; lower curve adrenalin (alkaloid) 1:100,000 solution was substituted between the arrows for the Ringer's. Reduced $\frac{1}{3}$.

SUMMARY

1. No difference was observed in the activity of the digestive systems of recently caught and starved frogs.
2. The longitudinal muscle of the esophagus is more sensitive to adrenalin or epinephrine than is the circular muscle. Weak solutions of adrenalin produce tonic contraction of both coats and strong solutions relaxation.
3. The weak solutions of adrenalin increase the tonus and in some cases the rate and force of contractions of both muscular coats of the frog's stomach, small intestine, rectum and cloaca. Stronger solutions produce relaxation in all these organs.

4. The action of adrenalin is the same upon what appears to be the cardiac and pyloric sphincters as it is upon the adjoining organs.

5. In some cases the action of adrenalin is the same upon the ileo-colic sphincter as upon the adjoining organs; in most cases, however, the sphincter responded to all concentrations by tonic contraction.

6. Strips taken from various parts of the frog's small intestine react to the same concentration of adrenalin in practically the same manner.

7. The lower part of the alimentary canal of the frog, the intestine, rectum and cloaca, appear to be more sensitive to adrenalin than the circular esophagus. They responded by contraction and relaxation to much weaker solutions than did the circular esophagus.

8. Adrenalin brought about spontaneous rhythmical contractions in some strips which had not been present and in others it caused the contractions to be increased in force and rate for long periods.

9. After the strips were bathed with adrenalin the tonus waves appeared to be more frequent than without the adrenalin.

10. The thoracic-lumbar autonomic nervous system must supply all the organs and sphincters except possibly the ileo-colic with both tonic augmentative and inhibitory nerve fibers. Weak solutions of adrenalin excite the tonic augmentative fibers, strong solutions of adrenalin excite the inhibitory fibers.

11. Changes in hydrogen-ion concentration of the Ringer's bath due to the addition of adrenalin chloride do not alter the reactions of the tissues.

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THE ACTION OF MORPHINE ON THE VOMITING CENTER IN THE DOG

CHAUNCEY D. LEAKE

From the Physiological Laboratory of the University of Wisconsin

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Textbooks in pharmacology note that in the dog morphine first stimulates and then depresses the respiratory center. Mayor is quoted as stating that morphine in the dog causes first a stimulation of respiration, in consequence of the nausea produced by the drug, and secondarily a depression (1). Cushny makes the same general observation (2). In Sollman's 1906 text, it is stated that after morphine "in the dog, the primary slowing of the respiration is almost invariably followed by a sudden large increase (the rate may rise in ten minutes from 24 to 234). This is again succeeded by slowing" (3).

These statements, in view of the well established fact that morphine reduces oxygen consumption, suggested that the propositions advanced by Gasser and Loevenhart (4) in regard to the effect of reduced oxidation on the vital centers may be operative following the administration of morphine.

In a series of experiments on the action of morphine on the dog, the effect of the drug on the vomiting center was studied in six animals, for which protocols are here presented. These dogs received a subcutaneous injection of 10 mgm. morphine sulphate per kilogram body weight, and all vomited within twelve minutes after the administration of the drug. In two animals, twice the effective dosage of emetine (a locally acting emetic) was injected subcutaneously five hours after the morphine. No vomiting or indications of nausea resulted. An hour later these same two animals received a subcutaneous injection of twice the effective dosage of apomorphine (a centrally acting emetic) with

TABLE 1

Healthy brown long-haired male dog, weight 11.2 kgm.

TIME	RESPIRA- TIONS PER MINUTE	PULSE- RATE PER MINUTE	REMARKS
11:10	30	108	Animal loose in kennel
11:15			Injected 10 mgm. morphine sulphate per kilo-gram
11:23	70	108	Restless, panting
11:25			Retching movements
11:27			Vomited
11:31	120	100	Pupils widely dilated
11:50	18	88	Quiet, pupils constricted
4:05	15	72	Subcutaneous injection 30 mgm. emetine. No response at all
5:00	20	88	Subcutaneous injection 10 mgm. apomorphine. No response within half hour, when observation ceased

TABLE 2

Healthy brown and black male dog, weight 20.2 kgm.

TIME	RESPIRA- TIONS PER MINUTE	PULSE- RATE PER MINUTE	REMARKS
9:50	24	112	Animal loose in kennel
10:00			Injected 10 mgm. morphine sulphate per kilogram
10:06	80	104	Restless
10:10	140	96	"Hyenoid gait" appears
10:12			Retching movements
10:13			Vomited
10:15			Defecated
10:20	210	88	Pupils widely dilated
10:30	160	76	Salivating freely
11:00	120	62	Quiet, lying down
11:30	40	48	Pupils constricted
2:30	20	60	
3:30	24	104	Subcutaneous injection 60 mgm. emetine. Seemed to give slight local irritation at point of injection. No further response
4:30	30	120	Subcutaneous injection 50 mgm. apomorphine. No reaction
5:30	20	120	Very deeply narcotized. Pulse irregular

no effect. In another animal the emetine was given two and a half hours after the morphine, and the apomorphine at the fourth hour, with no action from either drug. In two other dogs, apomorphine was administered at the second and third hours respectively after morphine, and emetine at the third and fourth hours respectively, without producing any emetic effect. In the

TABLE 3

Healthy black female toy-terrier, weight 4.1 kgm.

TIME	RESPIRA- TIONS PER MINUTE	PULSE- RATE PER MINUTE	REMARKS
8:30	16	80	Animal loose in kennel
8:35			Injected 10 mgm. morphine sulphate per kilogram
8:41	30	76	Retching and vomiting
8:45	38	76	Defecated
8:57	45	72	Pupils dilated
9:05	18	68	Quiet
9:15	14	60	
9:40	12	52	
10:56			Subcutaneous injection 15 mgm. emetine
11:10	14	60	No response to emetine
11:30	18	68	No response to emetine
11:40	18	68	Subcutaneous injection 12 mgm. apomorphine
11:55	14	72	No response to apomorphine. Very quiet, pulse irregular
12:15	16	72	No response to apomorphine. Pulse irregular, very deeply narcotized
10:00 next day	20	96	15 mgm. emetine. Vomited within fifteen minutes. No retching or distress
11:00 next day	20	96	12 mgm. apomorphine. Severe retching and vomiting within five minutes, lasting for ten minutes

remaining dog of the series, emesis following morphine occurred within ten minutes, and fifteen minutes after morphine was given, emetine was injected. Vomiting again resulted after eight minutes. Apomorphine was now administered, and within three minutes vomiting again followed. An hour later however, emetine had no effect, and at the second and third hours after, repeated injections of apomorphine had no emetic action.

This experiment suggested that fatigue of the vomiting center might have been the cause of the phenomena noted. Accordingly two dogs were given sub-minimal doses of morphine (0.3 mgm. per kilogram) every ten minutes until 6 mgm. per kilogram had been given. In these animals there was no stimulation of respiration at any time, and in only one any indication of nausea. Both defecated after receiving a milligram of the drug. Neither

TABLE 4

Brown short-haired male dog, good health, weight, 14.3 kgm.

TIME	RESPIRA- TIONS PER MINUTE	PULSE- RATE PER MINUTE	REMARKS
8:50	20	100	Animal loose in kennel
8:53			Injected 10 mgm. morphine sulphate per kilogram
9:00	36	92	Lying down
9:02	48	88	Vomited
9:15	120	72	Defecated, pupils widely open
9:20	212	60	Very quiet
9:30	48	60	Pupils constricted
9:50	18	56	Whining
10:45	28	48	Pulse irregular
11:20	28	56	Subcutaneous injection 40 mgm. apomorphine. Slight whining, and twitching of fore-legs
11:45	16	56	Pulse very irregular. Deeply narcotized. No response to apomorphine
12:20	16	78	Subcutaneous injection 50 mgm. emetine
1:20	20	82	No response to emetine
8:30 next day	20	118	40 mgm. apomorphine. Response within four minutes. Vomiting for ten minutes
9:30 next day	18	112	50 mgm. emetine. No response

vomited. In both respiration and pulse gradually diminished as deep narcosis came on. Four hours after the administration of morphine was begun, there was no response to twice the effective dose of emetine, and at the fifth hour, no effect from twice the active dose of apomorphine.

That fatigue of the vomiting center resulting from previous stimulation is not a prominent factor in causing the antemetic effects of the use of morphine was further shown by administering

emetine and apomorphine to a dog without previous injection of morphine. The animal responded to the normal dosage of emetine within fifteen minutes, and forty-five minutes later, when the usual dosage of apomorphine was injected, violent retching and vomiting began within three minutes and lasted intermittently for a half hour. After a two hour rest, during which period oatmeal water was given by mouth, apomorphine again caused violent retching and vomiting for twenty minutes.

TABLE 5

Black short-haired male dog, distemper, weight 5.5 kgm.

TIME	RESPIRATIONS PER MINUTE	PULSE-RATE PER MINUTE	REMARKS
8:00	18	80	Animal loose in kennel
8:05			Injected 10 mgm. morphine sulphate per kilogram
8:11	24		Restless
8:12			Vomited
8:20	32	72	Lying down, pupile dilated
8:30	90	68	Quiet
9:00	12	56	
11:00	12	66	Subcutaneous injection 15 mgm. apomorphine
11:30	11	60	Very deeply narcotized. No response to apomorphine
12:00	8	66	Subcutaneous injection 20 mgm. emetine
1:00	10	76	No response to emetine
11:00 next day	20	92	15 mgm. apomorphine. Response within a minute. Vomiting for fifteen
12:00 next day	20	90	20 mgm. emetine. Uneasiness, and indications of nausea, but no vomiting

Magnus (5), in 1906, noted an antemetic action of morphine, but attributed it to constriction of the cardiac sphincter. Post-mortem examination of morphinized dogs killed by injecting air into the jugular vein, however, revealed no constriction of the cardioesophageal junction.

It is suggested that the action of morphine in firstly stimulating and secondly depressing the vomiting center, together with its respiratory and vagal effects, may be explained by reference to the reaction to reduced oxidation, as a result of the drug, upon

the vital centers, as outlined by Gasser and Loevenhart (4). Experimental evidence that morphine has a special affinity for nervous, and especially brain, tissues, and that it is presumably destroyed by oxidation in these tissues, has been furnished by Cloetta (6). He found that brain tissues in vitro fix morphine better than any other tissues of the body, even liver, and that destruction of the morphine in these conditions, while not rapid, proceeds if the emulsified tissues are oxygenated, but not otherwise.

TABLE 6

Large brown male dog, good health, weight 13.6 kgm.

TIME	RESPIRA- TIONS PER MINUTE	PULSE- RATE PER MINUTE	REMARKS
11:55	24	114	Animal loose in kennel. Injected 10 mgm. morphine sulphate per kilogram
12:10	66	96	Retching movements, vomited
12:15	72	88	Subcutaneous injection 35 mgm. emetine
12:23	60	76	Vomited. No apparent distress
12:25			Subcutaneous injection 20 mgm. apomorphine
12:28			Severe retching, vomiting
1:00	18	92	35 mgm. emetine. No effect
2:00	18	104	20 mgm. apomorphine. No effect
3:00	16	104	40 mgm. apomorphine. No effect. Pulse irregular, and animal deeply narcotized

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NATURALLY NEPHROPATHIC ANIMALS

THE ABILITY OF AN ALKALINE SOLUTION TO INFLUENCE THE AMOUNT OF STAINABLE LIPOID MATERIAL THAT APPEARS IN THE KIDNEY FOLLOWING THE USE OF A GENERAL ANESTHETIC

WM. DEB. MACNIDER

Laboratory of Pharmacology, University of North Carolina

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In several papers (1, 2, 3) published some years ago, the observation was made that, when a solution of uranium nitrate was given to dogs subcutaneously, there appeared a deposition of stainable lipoid material in the epithelial cells of the loops of Henle and in the convoluted tubule epithelium. In a later publication (4) that was concerned with the protection of the normal kidney against the toxic effect of uranium nitrate by the use of a solution of sodium carbonate, the further observation was made that the control animals of the series showed a marked accumulation of stainable lipoid material in the cells of the loops of Henle and to a less extent in the convoluted tubule cells, while the carbonate or protected animals showed none of this material or a trace of such lipoid material in the cells of these portions of the tubule. More recently was undertaken a study (5) of the ability of an alkaline solution to protect the naturally nephropathic kidney against the toxic effect of such general anesthetic substances as ether, chloroform, and Gréhant's anesthetic. In this investigation the observation was incidentally made that associated with the ability of an alkali to lessen or prevent the toxicity of an anesthetic in so far as the kidney was concerned, there existed a relationship between the degree of protection conferred and the amount of stainable lipoid material in the renal epithelium. The kidneys of those animals which were protected

against the anesthetic showed less stainable lipid in the renal epithelium than did the kidneys of those animals in which no protection had been established by the use of an alkaline solution. During the past year a study (6) was made of the amount and distribution of stainable lipid material in the kidney in animals of different age periods both before and after the use of the general anesthetics. Observations of a similar character have been made on naturally nephropathic animals. A study of kidney tissue from the latter group of animals showed very clearly that such animals not only have a considerable amount of stainable lipid material in the cells of the loops of Henle, but that one of the earliest indications of injury to the convoluted tubule epithelium which develops secondarily to an injury to the glomeruli, is an accumulation of such material in the cells of this portion of the tubule. If an anesthetic be given to such animals, there is a further very rapid accumulation of stainable lipid material in the convoluted tubule epithelium and urine formation is greatly reduced or the animals become anuric. Such animals fail to respond to various diuretic solutions.

The present study is concerned with observations upon the ability of a solution of sodium carbonate to influence the amount of stainable lipid material normally present in the renal epithelial cells of naturally nephropathic animals and to study the influence of such a solution on the amount of stainable lipid material that develops in kidney epithelium during the use of ether as a general anesthetic. The study, furthermore, includes observations on the changes induced in the acid-base equilibrium of the blood from the use of such a solution in anesthetized and in unanesthetized animals and of the diuretic value of theobromine in naturally nephropathic animals that have been given an alkaline solution before the use of an anesthetic as contrasted with control animals that were not given such a solution.

Some years ago Pearce (7) and Ophüls (8), and later Dayton (9), pointed out that nephropathic processes comparable to such changes found in human material occurred in the dog's kidney. In two publications (10, 11) from this laboratory the prevalence of such injuries in the dog's kidney has been discussed, the ana-

tomical changes classified, and physiological studies conducted with the naturally nephropathic kidney. The pathology of the kidney in such naturally acquired chronic nephropathies consists of a primary injury to the glomeruli with the production of intracapillary and capsular changes leading to the development of a chronic glomerulonephropathy. The tubular epithelium in the early stages of such an injury shows but slight evidence of damage. One of the earliest indications of the injury to the epithelium is an increase in the amount of stainable lipoid material in the cells of the loops of Henle and the appearance of such material in the convoluted tubule epithelium. In the following experiments, animals with this type of renal injury have been employed.

TECHNIQUE OF EXPERIMENTS

Thirty-five naturally nephropathic animals have been used in this series of experiments. The animals were kept in metabolism cages and studied for five days before the commencement of any experimental interference. During this period the animals were fed on the routine laboratory diet of scraps of meat and a mixture of bread with cooked rice. The animals were given daily 500 cc. of water.

The urine was examined qualitatively for albumin with the heat and acetic acid test, the cold contact test with nitric acid, and by the contact test with Mayer's Reagent.¹ The centrifugalized urines were studied for casts. The reserve alkali of the blood was determined by the method of Marriott (12). The phenol-suphonephthalein test for renal function was conducted according to the technique devised by Rowntree and Geraghty (13). The control animals of the series were given intravenously 25 cc. per kilogram of a 0.9 per cent solution of sodium chloride. The animals that were employed in studying the influence of an alkaline solution on the amount and distribution of stainable lipoid mate-

¹ Mayer's reagent: Mercuric chloride 10 grams, sodium chloride 65 grams, and citric acid 25 grams. Dissolve in 500 cc. of hot water. The reagent is supposed to give a ring reaction with albuminous urine in ten minutes with as little as 0.0002 per cent albumin.

Influence of alkaline solution on

NUMBER OF EXPERIMENT	URINE ALBUMIN AND CASTS	R. pH	PHTHALEIN PER CENT, 2 HOURS	NaCl or Na ₂ CO ₃ SOLUTION, 25 CC. PER KILOGRAM	ANESTHETIC	URINE FLOW PER MINUTE, DROPS	R. pH
1. Control. NaCl, no ether	618 cc., trace of albumin casts	8.05	62	NaCl solution, 0.9 per cent	No anes- thetic		8.0
2. Control. NaCl, no ether	480 cc., trace of albumin casts	8.0	50	NaCl solution, 0.9 per cent	No anes- thetic		8.0
3. Control. NaCl, no ether	588 cc., trace of albumin. Few casts	8.1	55	NaCl solution, 0.9 per cent	No anes- thetic		8.05
4. NaCl, ether	492 cc., trace of albumin casts	8.0	54	NaCl solution, 0.9 per cent	Ether	8	7.95
5. NaCl, ether	510 cc., heavy trace of albu- min casts	7.9	50	NaCl solution, 0.9 per cent	Ether	2	7.85
6. NaCl, ether	402 cc., trace of albumin casts	8.0	60	NaCl solution, 0.9 per cent	Ether	4	7.95
7. Control. Na ₂ CO ₃ , no ether	712 cc., albumin casts	8.0	55	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	No anes- thetic		8.15
8. Control. Na ₂ CO ₃ , no ether	440 cc., trace of albumin. Few casts	8.0	50	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	No anes- thetic		8.2
9. Control. Na ₂ CO ₃ , no ether	718 cc., heavy, trace of albu- min casts	7.95	45	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	No anes- thetic		8.15
10. Protected. Na ₂ CO ₃ , ether	611 cc., heavy trace of albu- min casts	7.95	42	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	Ether	11	8.05

Stainable lipid in renal epithelium

DIURETIC SOLUTION	END OF FIRST HOUR OF EXPERIMENT	URINE FLOW PER MINUTE, DROPS	R. pH	END OF SECOND HOUR OF EXPERIMENT	URINE FLOW PER MINUTE, DROPS	R. pH.	AMOUNT AND DISTRIBUTION OF STAINABLE LIPOID MATERIAL IN KIDNEY
			8.05	Animal killed			Droplets of lipid in loops of Henle. Dust like particles in convoluted tubule epithelium
			8.0	Animal killed			Droplets and occasionally fused masses of lipid in loop of Henle. Fine particles in con- voluted tubule epithelium
			8.1	Animal killed			Droplets of lipid in loop of Henle. Dust like particles in convoluted tubule epithelium
heobromine, 1 per cent, 1 cc. per kilogram		6	7.9		1	7.9	Masses and droplets of lipoids in loop of Henle. Numerous droplets and dustlike parti- cles in convoluted tubule cells
heobromine, 1 per cent, 1 cc. per kilogram		0	7.85		0	7.8	Masses and droplets of lipid in loop of Henle. Cells dis- tended. Droplets of lipid in convoluted tubule cells
heobromine, 1 per cent, 1 cc. per kilogram		2	7.9		2	7.9	Masses and droplets of lipid in loop of Henle. Droplets in convoluted tubule cells
			8.05	Animal killed			No stainable lipid in loop of Henle. No stainable lipid in convoluted tubule cells
			8.1	Animal killed			No stainable lipid in cells of loop of Henle or in convo- luted tubule epithelium
			8.0	Animal killed			No stainable lipid in cells of loop of Henle or in convo- luted tubule epithelium
heobromine, 1 per cent 1 cc. per kilogram		12	7.95		16	7.9	Small droplets of lipid in loop of Henle. Dust like particles in convoluted tubule cells

Influence of alkaline solution on

NUMBER OF EXPERIMENT	URINE ALBUMIN AND CASTS	R. pH	PHTHALEIN PER CENT, 2 HOURS	NaCl or Na ₂ CO ₃ SOLUTION, 25 CC. PER KILOGRAM	ANESTHETIC	URINE FLOW PER MINUTE, DROPS	R. pH
11. Protected. Na ₂ CO ₃ , ether	494 cc., trace of albumin casts	8.0	40	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	Ether	7	8.1
12. Protected. Na ₂ CO ₃ , ether	1081cc., trace of albumin casts	8.0	62	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	Ether	20	8.15
13. Protected. Na ₂ CO ₃ , ether	576 cc., heavy trace of albu- min casts	7.95	62	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	Ether	12	8.2
14. Protected. Na ₂ CO ₃ , ether	742 cc., heavy trace of albu- min casts	7.95	50	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	Ether	10	8.2
15. Protected. Na ₂ CO ₃ , ether	511 cc., trace of albumin. Few casts	8.0	64	Na ₂ CO ₃ solution, equimolecular with 0.9 NaCl	Ether	16	8.2

rial in the renal epithelium were given intravenously 25 cc. per kilogram of a solution of sodium carbonate equimolecular with a 0.9 per cent sodium chloride solution. Following cocainization of the skin of the animal's leg the above mentioned solutions were injected through the large saphenous vein.

The thirty-five naturally nephropathic animals that were used experimentally may be divided into four groups, the basis for the division depending upon the type of experimental procedure undertaken in the respective groups. Six of the animals that constitute the first group are represented in the table by experiments 1, 2, and 3. These animals were given intravenously a 0.9 per cent sodium chloride solution, were not anesthetized, and at the end of a two hour period were killed without the use of an

stainable lipid in renal epithelium—concluded

DIURETIC SOLUTION	END OF FIRST HOUR OF EXPERIMENT	URINE FLOW PER MINUTE, DROPS	R. pH	END OF SECOND HOUR OF EXPERIMENT	URINE FLOW PER MINUTE, DROPS	R. pH	AMOUNT AND DISTRIBUTION OF STAINABLE LIPOID MATERIAL IN KIDNEY
Theobromine, 1 per cent, 1 cc. per kilogram		10	8.0		10	8.0	No stainable lipid in cells of loop of Henle or in convo- luted tubule epithelium
Theobromine, 1 per cent, 1 cc. per kilogram		28	8.05		20	8.0	No stainable lipid in loop of Henle. No stainable lipid in convoluted tubule epithe- lium
Theobromine, 1 per cent, 1 cc. per kilogram		12	8.0		3	7.9	Small droplets of lipid in loop of Henle. Dustlike particles in convoluted tubule epithe- lium
Theobromine, 1 per cent, 1 cc. per kilogram		14	8.1		10	8.0	No stainable lipid in loop of Henle or in convoluted tubule cells
Theobromine, 1 per cent, 1 cc. per kilogram		20	8.15		16	8.0	No stainable lipid in loop of Henle or in convoluted tubule cells

anesthetic. This group of animals serves as a control for the second group of animals which were also given a solution of sodium chloride and in addition were anesthetized by ether for two hours. Falling in this second group are six animals represented in the table by experiments 4, 5, and 6.

The third group of eleven animals, experiments 7, 8, and 9 of the table, were given intravenously a solution of sodium carbonate equimolecular with 0.9 per cent sodium chloride solution. The animals were not anesthetized. At the end of a two-hour period of observation these animals were killed without the use of an anesthetic. The animals serve as controls for the final and fourth group of animals that received a similar amount of sodium carbonate solution and were furthermore anesthetized with ether

for a period of two hours. Included in this last group are twelve animals: experiments 10, 11, 12, 13, 14, and 15 of the table.

In the groups of animals that received the sodium carbonate injections, but which were not anesthetized only observations on the reserve alkali of the blood could be made during the two-hour period of the experiments. In the groups of animals that received the same solutions and were then anesthetized, the following additional observations were made. Through a mid-line abdominal incision the bladder and ureters were exposed. Canulas were tied into each ureter in order to observe the flow of urine. A cannula was placed in the femoral vein and connected with a burette. Through this connection the animals were given at one period of the etherization 1 cc. per kilogram of a 1 per cent solution of theobromine. Observations on the flow of urine and reserve alkali of the blood were made at hour intervals during the course of the experiments.

At the termination of the experiments the kidneys were removed and thin pieces of tissue placed in 0.9 per cent sodium chloride solution to be used for frozen sections. Such tissue was not fixed in any way, but was at once frozen and the sections stained for lipoid material with Scharlach R. by Herxheimer's method. Other tissue from both kidneys was fixed in 10 per cent formaline, in Zenker's fluid and in corrosive-acetic and used for the routine histological study.

OBSERVATION ON THE NATURALLY NEPHROPATHIC ANIMALS
PRIOR TO THE COMMENCEMENT OF THE
EXPERIMENTS

The observations recorded for fifteen of the naturally nephropathic animals that are included in the table of experiments are representative of the entire number of animals used in the investigation. As will be observed from a study of the table of experiments, the daily output of urine by the respective animals varied from 402 cc. to 1081 cc. The urine from all of the animals contained albumin and casts. The amount of albumin in two of the animals was very small and was only detected by the use of

Mayer's reagent. The urine of one of these animals, experiment 11, with only a mere trace of albumin, contained numerous casts; and during a two-hour period the animal eliminated only 40 per cent of phenolsulphonephthalein. The elimination of phenolsulphonephthalein by the different animals was uniformly below the normal. The output of the dye varied from a minimum output of 35 per cent to a maximum output of 64 per cent.

Determinations of the alkali reserve of the blood in the different animals varied from a normal reading of 8.1 to a reading indicative of a distinct depletion in the alkali reserve of 7.9. In this series of animals there occurred an apparent relationship between the amount of albumin in the urine and the degree to which there had developed a depletion in the alkali reserve of the blood. In experiments 5, 9, 10, 13, and 14, the urine of the animals showed a heavy trace of albumin. In these animals the reserve alkali of the blood gave evidence of the greatest depletion for any of the thirty-five animals of the group. The readings varied from 7.95 to 7.9. In the remaining animals in which the reserve alkali of the blood varied from 8.1 to 8, the urine showed only a trace of albumin, and in two of these animals the presence of albumin was only detected by the use of a very sensitive test.

OBSERVATIONS ON NATURALLY NEPHROPATHIC ANIMALS FOLLOWING THE INTRAVENOUS INJECTION OF A SOLUTION OF 0.9 PER CENT SODIUM CHLORIDE. THE AMOUNT AND DISTRIBUTION OF STAINABLE LIPOID MATERIAL IN THE RENAL EPITHELIUM

Six of the animals, experiments 1, 2, and 3, were given intravenously 25 cc. per kilogram of a 0.9 per cent solution of sodium chloride. The animals were not anesthetized. At the end of two hours they were killed without the use of an anesthetic and kidney tissue obtained for the purpose of studying the amount and distribution of stainable lipoid material in the renal epithelium. These animals serve as a control group for the second group of animals that received a similar injection of sodium chloride solution, but were anesthetized by ether for a two-hour period. Following the injection of a sodium chloride solution in the con-

trol group of naturally nephropathic animals, two of the number, experiments 1 and 3, showed a slight reduction in the alkali reserve of the blood. In the animal of experiment 1, the alkali reserve was reduced from the normal of 8.05 to 8, and in the animal of experiment 2, from 8.1 to 8.05. At the termination of these experiments, two hours later, the alkali reserve of the blood had returned to the normal readings obtained prior to the injection of the sodium chloride solution. In the remaining four animals of this unanesthetized group the use of sodium chloride failed to induce even a temporary disturbance in the acid-base equilibrium of the blood.

At the end of two hours the kidneys from these animals were removed and frozen sections stained for lipid material with Scharlach R. In such naturally nephropathic animals that have not been subjected to the action of an anesthetic, stainable lipid is found in the cells of the loops of Henle in the form of droplets and rarely as fused masses. In the convoluted tubule epithelium such lipid material is found in a very much less amount in the form of fine particles, or as a finer dustlike deposit. These observations concerning the amount and distribution of stainable lipid material in renal epithelial cells of naturally nephropathic animals that have not been anesthetized confirm the observations made in a former publication (6).

The intravenous injection of 25 cc. per kilogram of a 0.9 per cent solution of sodium chloride into such animals does not affect the amount and distribution of stainable lipid material that is normally present in the renal epithelium of naturally nephropathic animals.

Six naturally nephropathic animals represented by experiments 4, 5 and 6 of the table were employed in the second group of experiments. These animals were given 25 cc. per kilogram of a 0.9 per cent solution of sodium chloride and immediately following the injections the animals were anesthetized by ether for a period of two hours.

As will be observed from a study of the experiments representative of this group and included in the table (experiments 4, 5, and 6), all of the animals of this group were diuretic at a period half

an hour following complete etherization. Even at this early period of the experiments the reserve alkali of the blood was reduced in all of the animals. In the animals of experiments 4 and 6, the reserve alkali had been reduced following the use of ether from 8 to 7.9. In the animal of experiment 5, with a reserve alkali of the blood of 7.9 prior to the anesthesia, the use of ether had reduced the reserve alkali to 7.85.

Following these initial observations the animals were given intravenously 1 cc. per kilogram of a 1 per cent solution of theobromine. In such animals in which the anesthetic had induced a reduction in the reserve alkali of the blood, the use of theobromine failed to increase urine formation. At the end of the first hour of the experiments, the animals of experiments 4 and 6 showed a reduction in urine formation. The flow of urine was reduced in the animal of experiment 4 from 8 to 6 drops per minute, and in the animal of experiment 6, from 4 to 2 drops per minute. The animal of experiment 5 that was forming only 2 drops of urine per minute at the commencement of the experiment had now become anuric. At this period of the experiment one hour after complete etherization, the reserve alkali of the blood in the majority of the animals had undergone a further depletion. In the animal of experiment 5 no further change had taken place in the reserve alkali determination from that made at the completion of the anesthesia. In the animals of experiments 4 and 6, the reserve alkali showed a further reduction from 7.95 to 7.9 in both animals.

The final observations made on this group of animals at the end of two hours show that there is no increase in urine formation as the experiments progress and no attempt on the part of the animals to reestablish a normal acid-base balance of the blood. The animal of experiment 5 remained anuric throughout the experiment, and at the termination of the two hour period the reserve alkali of the blood was depleted to 7.8. During the last hour of the experiments the urine flow in the animal of experiment 4 was reduced from 6 drops to 1 drop per minute. The reserve alkali of the blood remained unchanged from the reading at the end of the first hour of the experiment which was 7.9. Urine

formation by the animal of experiment 6 was 2 drops per minute at the end of the first hour of the experiment and remained unchanged at the end of the experiment. The reserve alkali of the blood failed to show a further depletion during the second hour of the experiment.

The naturally nepropathic animals of the second group as represented by experiments 4, 5, and 6 when given intravenously a solution of sodium chloride and anesthetized by ether remain diuretic for a period immediately following the etherization. Even at this early stage of the etherization there occurs a reduction in the reserve alkali of the blood. This reduction increases with the duration of the anesthesia. When such animals are given intravenously a solution of theobromine, urine formation fails to increase but, on the contrary, shows a reduction.

A study of frozen sections stained with Scharlach R. from the kidneys of these animals that had received an intravenous injection of sodium chloride solution and had then been anesthetized for two hours shows the following occurrence and distribution of stainable lipid material. When contrasted with the first and control group of animals that were not anesthetized, the second group shows that the amount of stainable lipid in the renal epithelium had been very greatly increased by the use of ether as an anesthetic. In the first or control group of animals stainable lipid material appeared as droplets and rarely as masses in the cells of the loops of Henle and as very fine dustlike particles in the cells of the convoluted tubules.

In the second group of animals that were anesthetized, stainable lipid material is found in the loops of Henle in fused masses which may obliterate the outline of the cells and obscure the nuclei. Many of the cells take uniformly the Scharlach R. stain. In the convoluted tubule epithelium the increase in amount of stainable lipid material is found in the form of well defined droplets, which occasionally fuse into masses.

The following conclusions are made from a study of these first two groups of animals:

1. Stainable lipid material is normally present in small amounts in the cells of the loops of Henle and in the convoluted tubule epithelium of naturally nephropathic animals.

2. The occurrence of such material in these locations of the tubule may not be associated with a reduction in the reserve alkali of the blood below the normal.

3. The intravenous administration of a solution of sodium chloride prior to etherization does not protect the kidney of the naturally nephropathic animal against the toxic effect of ether.

4. Following an anesthesia by ether for two hours in a naturally nephropathic animal that has received intravenously a solution of sodium chloride there occurs a very marked increase in the amount of stainable lipoid material not only in the cells of the loops of Henle but especially in the cells of the convoluted tubule epithelium.

5. Associated with such changes in the tubular epithelium there develops a reduction in the alkali reserve of the blood. Urine formation is reduced. The diuretic substance theobromine which is supposed to act as a diuretic by stimulating renal epithelium becomes ineffective.

6. Associated with such changes in the life of these cells that permits an accumulation of stainable lipoid material, the cells also show cloudy swelling and an edema in terms of vacuolation.

OBSERVATIONS ON NATURALLY NEPHROPATHIC ANIMALS FOLLOWING THE INTRAVENOUS INJECTION OF A SOLUTION OF SODIUM CARBONATE EQUIMOLECULAR WITH A 0.9 PER CENT SOLUTION OF SODIUM CHLORIDE. THE AMOUNT AND DISTRIBUTION OF STAINABLE LIPOID MATERIAL IN THE RENAL EPITHELIUM

Eleven animals are included in the third group of experiments. The details of the results obtained in these experiments are represented in the table by experiments 7, 8, 9. The animals of this group were not anesthetized. They serve as control experiments for the animals of the fourth and final group that were anesthetized for two hours. Both groups of animals were given intravenously 25 cc. per kilogram of a solution of sodium carbonate equimolecular with 0.9 per cent sodium chloride solution.

In the animals of the third group, experiments 7, 8, and 9, the reserve alkali of the blood was determined immediately fol-

lowing the use of the alkaline solution and at the end of a two hour period of observation. At this time the animals were killed without the use of an anesthetic and kidney tissue obtained for a study of the effect of an alkaline solution on the amount and distribution of stainable lipoid material in the renal epithelium. In this group of animals such observations can be made without having to interpret the part played by the anesthetic in such a consideration.

As will be observed from a study of the course of experiments 7, 8, and 9, following the intravenous injection of a solution of sodium carbonate, there occurs an increase in the reserve alkali of the blood above that which the naturally nephropathic animal had established as its normal. The reserve alkali of the blood of the animal of experiment 7, increased from 8 to 8.15, in the animal of experiment 8, from 8 to 8.2 and in the animal of experiment 9, from 7.95 to 8.15. During the following two-hour period the reserve alkali of the blood was not only not reduced below the normal, but it was not reduced to that point of depletion which these naturally nephropathic animals had established as their pathological normal.

Prior to the use of the alkaline solution the reserve alkali of the blood for the animal of experiment 7 was 8. Two hours later the reserve alkali was 8.05. In the animal of experiment 9, the reserve alkali of the blood was 7.95. Two hours after the use of the alkaline solution the reserve alkali of the blood was 8. The intravenous use of 25 cc. per kilogram of a solution of sodium carbonate equimolecular with 0.9 per cent sodium chloride solution in an unanesthetized naturally nephropathic animal not only reestablishes the acid-base equilibrium of the blood of the animal but over a two-hour period this equilibrium is maintained.

When the kidneys of such animals that have received an alkaline solution are studied for the amount and distribution of stainable lipoid in the renal epithelium, the following observations were made. In such animals stainable lipoid with Scharlach R. does not appear in the cells of the loops of Henle or in the cells of the convoluted tubule epithelium.

The observation was made in a previous publication (6) that animals with a chronic glomerulonephropathy showed the presence of stainable lipid material in the cells of the loops of Henle and to a less extent in the cells of the convoluted tubules. This observation has been confirmed by observations made during the present study in the naturally nephropathic animals of group one, experiments 1, 2, and 3. The further observation is made from a study of the kidneys of the third group of animals, experiments 7, 8, and 9, as follows. The use in an unanesthetized naturally nephropathic animals of a solution of sodium carbonate which reestablishes and maintains for a two-hour period the normal acid-base equilibrium of the blood, or increases this equilibrium to a point slightly in favor of the hydroxyl ion, causes the lipid material in the epithelium of the kidney to fail to stain by Scharlach R. Whether or not the use of such a solution has so changed the chemical character of the lipid material that it fails to stain, or whether such material has actually disappeared from the cells remains a question for future study.

Twelve animals are included in the fourth and final group. This group of animals is represented in the table by experiments 10, 11, 12, 13, 14, and 15. The animals of this group, like the animals of the former and control group, were given intravenously 25 cc. per kilogram of a solution of sodium carbonate equimolecular with a 0.9 per cent solution of sodium chloride. The animals of the group under discussion were anesthetized by ether for a period of two hours.

A study of the course of the fourth group of experiments as represented by the animals of experiments 10 to 15, inclusive, shows that all the animals remain freely diuretic following the development of a state of complete anesthesia. The flow of urine in the different animals has varied from 7 to 20 drops per minute. When this result is contrasted with the animals of experiment 4, 5, and 6 that received a similar amount per kilogram of 0.9 per cent sodium chloride solution and were also anesthetized, the observation is clear that in such naturally nephropathic animals a solution of sodium carbonate equimolecular with a 0.9 per cent solution of sodium chloride is of greater

diuretic value than is the sodium chloride solution. The animals that received the latter solution formed urine at the rate of 2 to 7 drops per minute.

Following the use of a solution of sodium carbonate there occurs in all of the animals an increase in the reserve alkali of the blood. In the animal of experiment 10, with an alkali reserve of 7.95, the use of the carbonate solution increased the reserve alkali to 8.05. In the animal of experiment 15 the reserve alkali was increased from 8 to 8.2. At this period of the experiments when the reserve alkali of the blood in all of the animals had been increased to normal or slightly beyond this point, the animals were given intravenously 1 cc. per kilogram of a 1 per cent solution of theobromine. The results in all of the experiments were constant. The use of such a solution increases the flow of urine. In experiment 10, the flow of urine was increased only from 11 to 12 drops per minute. In the animal of experiment 12 the urine flow increased from 20 to 28 drops per minute and in the animal of experiment 14, from 10 to 14 drops per minute.

These observations are in striking contrast with the results obtained from the use of theobromine in the second group of animals, experiments 4, 5, and 6. These animals received a 0.9 per cent solution of sodium chloride in place of the alkaline solution and were also anesthetized. The animals remained diuretic. In this group of animals the theobromine solution was of no diuretic value; and as the anesthesia progressed and induced a reduction in the reserve alkali of the blood, urine formation by the animals was greatly reduced or the animals became anuric. The fourth group of animals that received the alkaline solution were responsive to the diuretic effect of theobromine and remained diuretic throughout the experiment.

A further study of the animals of group four shows that by the end of the first hour of the anesthesia there had developed a reduction in the reserve alkali of the blood below the point reached immediately following the injection of the alkaline solution. In only two of the animals was this reduction sufficient to bring the alkali reserve of the animal to the point maintained prior to the beginning of the experiment. In the other animals of the group

the reserve alkali of the blood was maintained at the end of the first hour of the anesthesia at a point slightly in excess of the normal alkali reserve for these animals.

During the second hour of the anesthesia all of the animals that received a solution of sodium carbonate remained diuretic. The formation of urine by the respective animals varied from 3 to 20 drops per minute. Those animals, experiments 10 and 13, that showed during the course of the second hour of the anesthesia a reduction in the reserve alkali to a point slightly below the reserve alkali reading prior to the beginning of the experiment were the animals least diuretic at the termination of the experiments. At this period, the animal of experiment 10, with a reserve alkali of 7.9, was forming 6 drops of urine per minute. The animal of experiment 13 with an alkali reserve of 7.9 was forming 3 drops of urine per minute. In an anesthetized naturally nephropathic animal there would appear to be a relationship between the ability of the animal to maintain a normal alkali reserve of the blood and the functional response of the kidney.

At the termination of the two-hour period of anesthesia and prior to the death of the animals, the kidneys were removed and tissue obtained for the routine study.

Kidney tissue from this group of animals that had received intravenously an alkaline solution when stained for lipid material with Scharlach R. show that, excepting two animals of the group, the use of ether as an anesthetic has not induced such changes in the life processes of the renal epithelial cells as to cause the deposition of stainable lipid material. In all of the experiments, except experiments 10 and 13, there was no stainable lipid material in the cells of the loops of Henle or in the cells of the convoluted tubules. During the course of the anesthesia in the animals of this group other than the animals of experiments 10 and 13, the reserve alkali of the blood had not been depleted below 8. In the animals of experiments 10 and 13 the reserve alkali of the blood had not only been depleted during the course of the anesthesia below this point but to a point below the normal for these naturally nephropathic animals before the beginning of the experiments. In these two animals stainable lipid was pres-

ent in the cells of the loops of Henle as well defined droplets and in the convoluted tubule epithelium as dustlike particles.

When a solution of sodium carbonate equimolecular with 0.9 per cent sodium chloride is given to naturally nephropathic animals, and the animals then anesthetized with ether, the use of the alkaline solution not only protects the kidney against the toxic effect of the anesthetic, but it influences the amount of stainable lipid material in the renal epithelium. If the alkali reserve of the blood is maintained during the period of anesthesia by the use of such a solution, stainable lipid material can not be demonstrated in the renal epithelial cells by Scharlach R. Such animals are freely diuretic at the termination of the experiments. When, however, such a solution is given to a naturally nephropathic animal, and during the course of a two hour period of anesthesia the reserve alkali of the blood is depleted to or below the point normal for the naturally nephropathic animal, stainable lipid material can be detected by Scharlach R. in the cells of the loops of Henle and in the convoluted tubule epithelium. Such animals have shown a reduction in urine formation during the course of the experiments.

CONCLUSIONS

1. The use of a solution of sodium carbonate in naturally nephropathic animals causes an increase in the reserve alkali of the blood which may be maintained in an unanesthetized animal over a period of two hours at a point above the normal alkali reserve determination for the animal.

2. A study of kidney tissue from such animals stained for lipid material with Scharlach R. by Herxheimer's method shows an absence of such material in the cells of the loops of Henle and in the convoluted tubule epithelium. Naturally nephropathic animals that have served as control experiments for these animals and have received intravenously a solution of sodium chloride equimolecular with the sodium carbonate solution have shown stainable material not only in the cells of the loops of Henle but also in the convoluted tubule epithelium. The deduction is made

that the use of a solution of sodium carbonate in a naturally nephropathic animal either so changes the character of the stainable lipoid material normally present in these cells so that it fails to stain with Scharlach R. or that the solution causes the disappearance of such material from these cells.

3. When naturally nephropathic animals are given a solution of sodium chloride and then anesthetized with ether for a two-hour period, the animals are found to be unable to maintain during the anesthesia a normal alkali reserve of the blood. There occurs a reduction in the alkali reserve of the blood and urine formation is reduced or the animals become anuric.

4. When naturally nephropathic animals are given intravenously a solution of sodium carbonate and then anesthetized with ether for a two-hour period, it has been found that ten of the twelve animals which have been subjected to such a technique have maintained during the course of the anesthesia a blood which has failed to show a depletion in the alkali reserve below the normal. The kidneys of such animals have failed to show the presence of stainable lipoid material in the cells of the loops of Henle or in the convoluted tubule epithelium. The animals have remained freely diuretic and responsive to a diuretic substance, theobromine, that is supposed to exert its diuretic effect through stimulating the epithelial mechanism in the kidney.

Two of the twelve animals that received intravenously a solution of sodium carbonate and were then anesthetized were unable to maintain during the period of anesthesia a normal acid-base equilibrium of the blood. The blood showed a depletion in the alkali reserve below the point established as a normal by the naturally nephropathic animals. Kidney tissue from these animals has shown lipoid material stainable with Scharlach R. in the cells of the loops of Henle and in the convoluted tubule epithelium. Such animals were less diuretic than were animals in which stainable lipoid material could not be demonstrated in the renal epithelium. The kidneys of these animals either failed to respond to the diuretic effect of theobromine, or the response was slight in comparison with that obtained in the other animals of the group.

5. The experiments not only show the ability of a solution of sodium carbonate to influence the amount of stainable lipid material in the renal epithelium, but they also indicate a relationship between the amount of stainable lipid material in the renal epithelium and the toxicity of the anesthetic for the kidney.

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PICROTOXIN HYPERGLYCEMIA

ARTHUR L. TATUM

From the Laboratory of Pharmacology of the University of Chicago, Chicago

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There are many drugs which affect the sugar equilibrium in the body, yet the mechanism of action of none is satisfactorily known. The neurogenic and chemical theories of control have not been entirely established nor clearly separated. According to current thought a drug may act on sugar storage cells directly, or indirectly through chemical changes in the blood subsequent to drug action or may act centrally in a manner analogous to the piquê injury.

An analysis of this problem may likely be rendered more certain with an increase of information obtained from a study of different agencies. In a previous study along this line we had occasion to employ picrotoxin as a central vagal stimulant, and found to our then embarrassment that picrotoxin itself was a powerful glycogenolytic agent. This observation led us to further investigate this drug and its mode of action, the results of which study are herein reported.

So far as we have been able to find, this action of picrotoxin has not been reported. Among the convulsants the caffeine series and strychnine have been most studied, the former being effective in nonconvulsant doses, the latter in convulsant doses and both probably ineffective after double splanchnotomy.

In table 1 there is seen a marked rise in blood sugar concentration in doses which were too small to produce convulsions and also in doses just large enough to produce mild but not dangerous convulsions. The response in terms of sugar values was of a high order relative to the quantity of picrotoxin injected, being about one-tenth as powerful as epinephrine milligram for milligram.

TABLE 1

The effect of subcutaneous injections of picrotoxin on blood sugar and alkaline reserve capacity of whole blood† in normal rabbits*

ANIMAL NUMBER	TIME	BLOOD SUGAR	ALKALINE RESERVE CAPACITY	NOTES
	1921	mg./cc.	cc.	
32	March 8, 2:00 p.m.	0.94		
	2:24 p.m.			3 mgm. picrotoxin
	2:50 p.m.			Heart slowed, hyperpnea
	3:00 p.m.	2.51		
	3:05 p.m.			Convulsion
44	March 9, 11:00 a.m.	1.08	0.80	
	11:20 a.m.			1 mgm. picrotoxin
	12:15 p.m.	1.08	0.82	
45	March 11, 11:00 a.m.	1.08	0.78	
	11:54 a.m.			2 mgm. picrotoxin
	1:00 p.m.	1.08	0.81	
	1:55 p.m.			2 mgm. picrotoxin
	2:17 p.m.	1.88	0.79	
	3:00 p.m.	3.23	0.73	
	4:30 p.m.	3.23	0.68	
46	April 12, 1:45 p.m.	0.90	0.76	
	2:09 p.m.			4 mgm. picrotoxin
	2:35 p.m.	1.03	0.70	
	2:50 p.m.			Convulsions
	5:00 p.m.	0.66		Convulsions ceased
	5:28 p.m.			0.5 cc. adrenalin
	6:05 p.m.	1.26		
47	April 13, 11:00 a.m.	1.19	0.62	
	1:00 p.m.		0.65	
	1:19 p.m.			3 mgm. picrotoxin
	1:35 p.m.			Heart slowed, irregular
	1:40 p.m.	1.61	0.58	
	2:30 p.m.	2.83	0.52	
	4:30 p.m.	2.26	0.53	
48	April 16, 11:00 a.m.	1.25	0.76	
	11:33 a.m.			3 mgm. picrotoxin
	12:18 p.m.	2.15	0.70	
	12:25 p.m.			Slight convulsion
49	April 16, 3:20 p.m.	0.80	0.71	
	3:38 p.m.			3 mgm. picrotoxin
	4:10 p.m.	1.12	0.70	
	4:40 p.m.	1.26	0.71	

TABLE 1—*Continued*

ANIMAL NUMBER	TIME	BLOOD SUGAR	ALKALINE RESERVE CAPACITY	NOTES
	<i>1921</i>	<i>mg./cc.</i>	<i>cc.</i>	
50	April 18, 9:45 a.m.	1.13	0.85	
	10:15 a.m.		0.84	
	10:40 a.m.			2 mgm. picrotoxin
	11:10 a.m.	1.76	0.73	
	11:40 a.m.	2.83	0.66	
51	April 19, 10:30 a.m.	0.98	0.82	
	11:00 a.m.			2 mgm. picrotoxin
	11:20 a.m.	1.13	0.80	
	11:50 a.m.	1.88	0.78	
52	April 20, 2:10 p.m.	1.03	0.79	
	2:40 p.m.			2 mgm. picrotoxin
	3:10 p.m.	1.19	0.78	
	3:40 p.m.	2.04	0.77	
	April 21, 10:45 a.m.	0.87	0.70	
	11:08 a.m.			4 mgm. picrotoxin
	11:40 a.m.	1.81	0.73	
	12:00 m.			Slight convulsion
54	1:45 p.m.	2.15	0.78	
	May 4, 2:15 p.m.	0.90	0.67	
	2:32 p.m.			4 mgm. picrotoxin
	3:02 p.m.	1.41	0.60	
	3:15 p.m.			Violent convulsion
55	3:45 p.m.			Animal died
	May 5, 9:50 a.m.	1.03	0.67	
	10:16 a.m.			3 mgm. picrotoxin
	10:50 a.m.	1.26	0.64	
	11:20 a.m.	1.41	0.60	
56	May 6, 10:00 a.m.	0.98	0.77	
	10:27 a.m.			3 mgm. picrotoxin
	11:00 a.m.	1.33	0.76	
	11:40 a.m.	2.51	0.75	
57	May 9, 10:15 a.m.	1.13	0.66	
	10:42 a.m.			3 mgm. picrotoxin
	11:13 a.m.	1.41	0.63	
	11:50 a.m.	2.83	0.63	

* Blood sugar analyses were made by the Benedict method.

† Alkaline reserve capacity of whole blood was determined by an adaptation of Van Slyke's method. Uncorrected values.

Tatum, A. L., Jr., Biol. Ch. 1920, xli, 59.

The alkaline reserve values frequently fell, but not constantly, and occasionally there occurred a marked sugar change with no change in reserve values. This may be taken as additional evidence that reserve changes in the circulatory blood is no certain criterion of glycogenolysis. Animals receiving effective picrotoxin dosages invariably showed marked vagal slowing of the heart, which might be thought to be a contributory or causative factor on the basis of circulatory disturbances subsequent to central vagus stimulation. This factor will be proven to be entirely eliminated in a subsequent section of this paper, in which it is found that the vagal factor plays no significant rôle.

The next question naturally arising is as to the point of action of picrotoxin as a glycogenolytic agent, whether it is central or peripheral. Picrotoxin has long been known to be a central nervous system stimulant affecting preeminently the medulla and midbrain regions of the brain stem. By some it is spoken of as a stimulant of the central nuclei of the parasympathetic nervous system. Consequently one is entirely justified in anticipating that the glycogenolytic action is also due to a central disturbance of what we choose to call the *glycotaxic* mechanism.

From the table 2 it is to be seen that in double splanchnotomy, regardless of time following the operation, picrotoxin completely fails to produce a detectible change in sugar concentration. These animals were in good physical condition, well nourished, lively and in all respects apparently entirely normal. These same animals gave normal quantitative responses to epinephrine in sugar changes, consequently the liver could not be said to be devoid of nor even noticeable deficient in hepatic glycogen.

This set of observations unequivocally localizes the action of picrotoxin in the nervous system central to the point of splanchnic nerve section by laparotomy. A closer localization of the point of action has not particularly concerned us.

This set of experiments also proves that central stimulation of the vagus center with its subsequent cardiac slowing plays no essential or primary rôle, for in these animals as well as in normals, the heart was almost invariably markedly slowed. Respiration was also usually increased in both operated and normal animals.

TABLE 2

The effect of subcutaneous injections of picrotoxin on blood sugar and alkaline reserve capacity of whole blood in animals previously subjected to double splanchnotomy

ANIMAL NUMBER	WEIGHT	TIME		BLOOD SUGAR	ALKA- LINE RE- SERVE	NOTES
	<i>kgm.</i>	<i>1921</i>		<i>mg./cc.</i>	<i>cc.</i>	
65	1.9	July 15,	11:00 a.m.	1.10	0.66	
			11:20 a.m.			
			12:10 p.m.	1.16	0.68	3 mgm. picrotoxin
66	2.7	July 19,	9:15 a.m.	1.03	0.62	
			9:45 a.m.			
			10:30 a.m.	1.03	0.62	3 mgm. picrotoxin
		July 30,	11:00 a.m.	1.33		
			11:12 a.m.			4 mgm. picrotoxin
			12:00 m.			1 mgm. picrotoxin
			12:22 p.m.	1.33		
		August 2,	2:00 p.m.	1.41		
			2:04 p.m.			0.5 cc. adrenalin
			3:04 p.m.	4.50		
65	1.9	August 1,	2:20 p.m.	1.33		
			2:22 p.m.			0.5 cc. adrenalin
			3:25 p.m.	3.76		
84	1.6	November 14,	9:30 p.m.	1.08		
			9:35 p.m.			0.5 cc. adrenalin
			10:30 p.m.	2.82		
		November 16,	9:50 p.m.	1.19		
			10:15 p.m.			2 mgm. picrotoxin
			11:15 p.m.	1.13		
		November 24,	10:00 p.m.	1.26		
			10:15 p.m.			0.5 cc. adrenalin
			11:15 p.m.	2.82		
85	2.2	November 16,	3:00 p.m.	1.10		
			3:15 p.m.			2.4 mgm. picrotoxin
			4:15 p.m.	1.13		
		November 24,	2:45 p.m.	1.19		
			2:52 p.m.			0.5 cc. adrenalin
			3:52 p.m.	2.80		
86	2.0	November 17,	10:00 p.m.	1.10		
			10:15 p.m.			2.4 mgm. picrotoxin
			11:15 p.m.	1.10		
87	2.2	November 17,	2:50 p.m.	0.80		Unoperated control
			2:57 p.m.			2.4 mgm. picrotoxin
			4:00 p.m.	1.88		

The next question pertinent to our study was that of the rôle played by the adrenal glands in this centrally induced disturbance in the glycotaxic mechanism. This we have attempted to answer by adrenal gland denervation. To accomplish this we sectioned the right splanchnic nerve and removed the left adrenal gland with as little trauma to nerves about the adrenal gland as possible. A similar adrenalectomy operation we have previously found did not abolish vasomotor action from splanchnic nerve stimulation on the same side, so we feel that a considerable amount of the splanchnic nerve was not injured. We were aware of Nishi's¹ observations from which he concluded that the left splanchnic nerve supplies the right adrenal gland as well as the left, and should the results of our study confirm Nishi we would have proceeded to reverse the operation.

As seen in table 3, there was complete absence of glycogenolytic response as indicated by changes in concentration of blood sugar. Obviously whether or not sugar changes occur not detectible by a study of concentration, we are unprepared to state.

The conclusion one is forced to concede is that this operation as well as double splanchnotomy abolish the centrally induced action of picrotoxin as a glycogenolytic agent. This strongly indicates the necessary intervention of the adrenal glands in the completed process. We recognize that nerve trauma inevitably occurs in adrenalectomy and cannot deny the possibility of so-called glycogenolytic nerve fibers passing via the bundle of fibers in the adrenal adnexa being injured by the operation. However, we have attempted hulling out of adrenal tissue from the capsule, and with results like those of complete excision of the gland with its adherent capsule. We have no reason for denying the occurrence of glycogenolysis of central induction in the absence of adrenal glands as shown by Stewart and Rogoff² by piqure, but do state that the action of picrotoxin, in our hands, is of such an order of intensity as to require the intact adrenal gland mechanism to be effective. We feel that the evidence indicates that the adrenal glands do mediate this centrally induced glycogenolysis,

¹ Nishi, M., Arch. f. exp. Path. u. Pharmak., 1909, lxi, 401.

² Stewart, G. N. and Rogoff, J. M., Am. Jour. Physiol., 1918, xlvii, 90.

TABLE 3

The effect of subcutaneous injections of picrotoxin on blood sugar and alkaline reserve capacity of whole blood in animals previously subjected to right splanchnotomy and left adrenalectomy

ANIMAL NUMBER	WEIGHT	TIME		BLOOD SUGAR	ALKA- LINE RE- SERVE	NOTES
	<i>kgm.</i>	<i>1921</i>		<i>mgm. cc.</i>	<i>cc.</i>	
67	3.0	July 20,	8:20	1.19	0.66	3 mgm. picrotoxin
			8:40			
			9:40	1.08	0.68	
			4:55			0.5 cc. adrenalin
			5:55	2.26	0.63	
68	2.6	July 21,	10:30	1.03		3 mgm. picrotoxin
			10:45			
			11:50	1.03		
		July 31,	8:45	1.33		3 mgm. picrotoxin
			9:00			
			10:00	1.18		
		August 1,	8:10			0.5 cc. Adrenalin
			9:10	3.76		
69	1.7	July 23,	9:45	1.13		2 mgm. picrotoxin
			9:58			
			10:58	1.03		
		August 4,	9::30	1.41		2 mgm. picrotoxin (Mild convulsion)
			9:36			
			10:00	1.19		
		August 4,	1:13			0.5 cc. adrenalin
			2:13	3.76		
70	1.8	August 5,	9:00	1.50		2 mgm. picrotoxin
			9:08			
			10:10	1.33		
71	2.0	August 22,	8:50	1.13		2 mgm. picrotoxin
			8:58			
			10:00	0.87		
72	2.0	August 22,	1:15	0.98		2 mgm. picrotoxin
			1:20			
			2:20	0.94		

TABLE 3—*Concluded*

ANIMAL NUMBER	WEIGHT	TIME	BLOOD SUGAR	ALKA- LINE RE- SERVE	NOTES
	<i>kgm.</i>	<i>1931</i>	<i>mgm. cc.</i>	<i>cc.</i>	
91	2.0	December 19, 10:00	1.18		
		10:15			
		11:15	1.18		2 mgm. picrotoxin (Evidences of ap- proaching spasm)
92	2.2	December 22, 2:00	1.25		
		2:10			
		3:10	1.25		2 mgm. picrotoxin
93	2.2	December 23, 10:45	1.41		
		10:52			
		11:52	1.41		2 mgm. picrotoxin

but we do not contest the proposition that if sufficiently intense or adequate, a central irritation can produce glycogenolysis in the absence of adrenal gland participation.

In regard to Nishi's contention that the left splanchnic nerve in the rabbit innervates the right as well as the left adrenal gland we can only state that we have met with no evidence supporting this view, hence were under no necessity of reversing the operative procedure, namely, left splanchnotomy and right adrenalectomy.

CONCLUSIONS

1. Picrotoxin is a centrally acting glycogenolytic agent effective in subconvulsant dosage.

2. Picrotoxin does not act to produce hyperglycemia in animals with denervated adrenal glands which fact indicates their necessary intervention in the production of picrotoxin hyperglycemia.

THE ACTION OF QUININE ON SUGAR MOBILIZATION WITH ITS BEARING ON THE QUESTION OF GLYCOGENOLYSIS

A. L. TATUM AND R. A. CUTTING

From the Laboratory of Pharmacology of the University of Chicago, Chicago

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Epinephrine as is well established produces rapid lysis of hepatic glycogen by peripheral action presumably by affecting the terminations of nerves from the hepatic plexus. In a preceding paper one of us has reported results of a study of picrotoxin¹ which effects glycogenolysis by central stimulation of sympathetic neurones whose efferent paths lie in the splanchnic nerves. Since any glycogenolytic agent may, as a working hypothesis, operate by some type of alteration of metabolism, the proposition is near to study the effects of a drug with recognized effects on cellular metabolism. Quinine most naturally suggests itself since it is generally known to depress metabolism, and particularly that of protein. Indeed a beginning of this study was made by Silberstein² in 1915, who reported hyperglycemia in dogs after administration of quinine. Silberstein presented no evidences regarding the mode of action of quinine nor of the conditions under which it may act. In view of the possibilities of such a study giving further light on glycogenolysis regardless of the provocative agency, it was considered feasible to repeat the work of Silberstein and to extend the study.

Rabbits were used exclusively in this study, blood sugar determinations were made partly by the Folin-Wu and partly by the Benedict methods as specified in the tables. Blood was taken from the marginal ear vein, both for sugar and in part of the in-

¹ Tatum, A. L., Jour. Pharm. and Exp. Therap. (in press).

² Silberstein, F., Centralbl. f. physiol., 1915, xxix, 413.

TABLE 1

The effect of intramuscular injections of quinine on the concentration of blood sugar† in the normal rabbit*

ANIMAL NUMBER	TIME	BLOOD DRAWN	BLOOD SUGAR	QUININE INJECTED
	1921	cc.	mgm./cc.	mgm./kilo.
1	November 5, 11:30 a.m.	2.5	0.96	100
	11:33 a.m.			
	1:28 p.m.	2.5	1.38	
2	November 5, 1:40 p.m.	2.5	0.74	100
	1:46 p.m.			
	3:50 p.m.	3.0	1.14	
3	December 17, 2:20 p.m.	2.5	1.40	100
	2:24 p.m.			
	4:28 p.m.	3.0	1.46	
4	December 24, 12:10 p.m.	3.0	0.99	100
	12:15 p.m.			
	2:08 p.m.	2.5	1.42	
5	December 24, 12:35 p.m.	2.5	1.00	100
	12:40 p.m.			
	2:41 p.m.	2.5	1.00	
6	December 24, 1:05 p.m.	3.0	1.00	100
	1:10 p.m.			
	3:00 p.m.	3.0	2.38	
7	December 24, 1:25 p.m.	2.5	1.21	100
	1:28 p.m.			
	3:31 p.m.	2.5	1.50	
8	December 24, 1:49 p.m.	2.5	1.11	100
	1:51 p.m.			
	3:50 p.m.	2.5	1.11	
	1922			
9	January 7, 10:40 p.m.	3.0	1.33	100
	10:45 p.m.			
	12:50 p.m.	3.0	2.17	
10	January 7, 11:25 p.m.	2.5	1.23	100
	11:28 p.m.			
	1:31 p.m.	2.5	1.71	

TABLE 1—*Continued*

ANIMAL NUMBER	TIME	BLOOD DRAWN	BLOOD SUGAR	QUININE INJECTED
	1922	cc.	mgm./cc.	mgm./kilo.
11	January 14, 12:40 p.m.	2.5	1.31	100
	12:42 p.m.			
	1:41 p.m.	2.5	2.12	
	1921			
22	November 26, 11:38 p.m.	3.0	1.34	200
	11:44 p.m.			
	1:44 p.m.	3.5	1.84	
23	December 9, 4.40 p.m.	2.5	0.80	200
	4:44 p.m.			
	6:50 p.m.	2.5	0.95	
	1922			
24	January 7, 12:00 p.m.	3.0	1.04	50
	12:03 p.m.			
	2:10 p.m.	2.5	1.04	
25	January 14, 11:30 p.m.	3.0	1.42	75
	11:33 p.m.			
	1:35 p.m.	3.0	1.19	
26	January 14, 12:03 p.m.	2.5	1.03	75
	12:05 p.m.			
	2:07 p.m.	3.0	1.29	

* The drug used for the greater part of this work was the product of the New York Quinine and Chemical Works, New York. During the course of the experiments the product of one other firm was used for a number of experiments, but this product was found to produce results similar to the above only in doses too large to be used with safety.

† Blood sugar analyses reported in tables 1, 2, and 3, were made by the method of Folin-Wu. Those in table 4, by Benedict's method.

vestigation for alkaline reserve capacity of whole blood using an adaptation of the Van Slyke method as previously described by one of us.³

Having obtained convincing evidences that quinine is a glyco-
genolytic agent, the question immediately pertinent is as to the
point of action, whether it acts on glycogen-containing cells di-
rectly as is suggested from the well known depression of metab-

³ Tatum, A. L., Jour. Biol. Ch., 1920, xli, 59.

olism, or whether it could be a central action on what we have termed the central glycotaxic mechanism.

If the action is peripheral it is to be expected that hyperglycemia should result even after double splanchnotomy. Epinephrine, we may state, is as efficient after double splanchnotomy as in normal animals. If the action is central, double splanchnotomy should abolish the glycogenolytic action of quinine as it does that of picrotoxin.

In table 2 it is seen that quinine glycogenolysis is quite effectively abolished by a previous double splanchnotomy. That these animals were glycogen free or even with a low supply of hepatic glycogen is rather strongly disproved by either prior or subsequent control testing by epinephrine. In these animals, epinephrine was as efficient as a glycogenolytic agent, as in normal animals. We believe we are justified in concluding from the results of tables 1 and 2, that hyperglycemia is produced by quinine affecting some central nervous system mechanism, whose disturbance leads, via splanchnic nerves, to glycogenolysis.

Additional strong evidence of the central action of quinine as a glycogenolytic agent is the rather frequent occurrence of a distinct and occasional high grade *hypoglycemia* in the double splanchnotomy series, as also in the series of denervated adrenals given below. This we take to be the peripheral reaction to quinine so far as concerns glycogen containing cells, namely a diminution of the capacity of such cells to transform glycogen into dextrose.⁴ The central action we take to be the initiation of lytic impulses which predominate over the antilytic state of the liver cells. The threshold values for both types of quinine action appear to be nearly the same for we were unable to find a dosage that would certainly produce a hypoglycemia in intact normal animals. In normal animals the minimal effective dosage almost invariably produced hyperglycemia, while in splanchnotomized animals essentially the same dosage was required to produce hypoglycemia.

⁴ Pick, Cavazzani, Iwanoff. Ref. by Heffter, Handbuch d. exp. Pharmak., ii, 1, p. 28.

TABLE 2

The effect of intramuscular injections of quinine on the concentration of blood sugar in splanchnotomized rabbits

ANIMAL NUMBER	TIME	BLOOD DRAWN	BLOOD SUGAR	QUININE INJECTED
	1921	cc.	mgm./cc.	mgm./kilo.
12	November 5, 2:30	3.0	1.40	100
	2:35			
	4:40	2.5	1.60	
13	November 5, 2:35	3.0	1.31	100
	2:38			
	4:35	2.5	1.27	
14	November 12, 10:50	2.5	1.36	100
	10:58			
	12:58	2.5	1.34	
15	November 19, 10:55	2.5	1.13	100
	11:00			
	1:23	3.5	1.05	
16	November 19, 11:15	2.5	1.25	100
	11:20			
	1:40	3.0	1.18	
17	November 19, 11:30	2.5	1.03	100
	11:32			
	1:43	3.0	1.07	
18	December 31, 1:10	3.0	1.14	100
	1:14			
	3:11	3.0	1.13	
19	December 31, 1:45	2.5	1.07	100
	1:50			
	3:48	3.0	0.87	
20	December 31, 2:15	3.0	1.18	100
	2:20			
	4:25	4.0	1.06	
21	December 31, 2:51	3.0	0.99	100
	2:54			
	4:53	3.0	0.85	

In regard to the rôle played by adrenals in the centrally induced hyperglycemia by quinine we may cite the results of experiments presented in table 3.

From table 3 it is to be observed that again quinine fails, in the dosages employed, to produce hyperglycemia. The dosages given were essentially the same as were found to be effective in producing hyperglycemia in normal animals. The precise rôle played by the adrenal glands we are not in a position to state. It may be (a) coöperative action of a secretion of adrenal glands with sympathetic impulses, (b) nervous relay involved in the adrenal glands or (c) nerve pathways lying in the adnexa of the adrenal glands.

TABLE 3

The effect of intramuscular injection of quinine on the concentration of blood sugar in animals subjected to left adrenalectomy and right splanchnotomy

ANIMAL NUMBER	TIME		BLOOD DRAWN	BLOOD SUGAR	QUININE INJECTED
	1922		cc.	mgm./cc.	mgm./kilo.
41	January 14,	12:29	2.5	1.08	100
		12:34			
		2:35	3.0	0.97	
42	January 14,	1:00	3.0	0.87	100
		1:04			
		3:03	3.0	0.86	

So far as our data permits of conclusions, we believe that the adrenal glands are in some way essential to the glycogenolytic efficiency of quinine in the dosages used and in animals in such conditions as they were in at the time of study. So far as epinephrine hyperglycemia is a fair criterion of hepatic glycogen, there was no evidence that the animals were deficient in glycogen. Resort was not made to artificial enrichment in sugar because that is in itself an additional abnormal state and not necessary for the conclusions drawn in this paper. It is possible that in livers surcharged with sugar, quinine might produce hyperglycemia in the absence of adrenal intervention as has been found following piqure by Stewart and Rogoff.⁵

⁵ Stewart, G. N., and Rogoff, J. M., Am. Jour. Physiol., 1918, xlv, 90.

Furthermore the evidences are good that adrenal denervation does not lead to any glycogen deficiency. In regard to the possibility of injury to lytic nerves by the unilateral adrenalectomy we may cite again Stewart and Rogoff who found pique to be effective in conditions of denervated adrenal glands especially in animals whose glycogen supply was artificially enriched. This indicates the existance of *some* lytic nerves after the operative procedure of adrenalectomy. In addition we have abundant evidence that removal of an adrenal does not abolish vasomotor responses from splanchnic nerve stimulation on that side proving the integrity of some fibers of the splanchnic, but obviously does not prove the intactness or integrity of glycogenolytic fibers which might be singled out for injury in the operation.

Having established to our own satisfaction the fact that quinine produces hyperglycemia by virtue of a central disturbance of the glycotaxic mechanism and this in some ill defined way intermediated by the normally innervated adrenal glands, we sought to compare the actions of quinine and of epinephrine. Epinephrine, as is well established, produces under ordinary conditions hyperglycemia concomitant with and as we believe from previously reported evidences,⁶ independent changes in acid-base balance in the body,—in particular, a fall in the alkaline reserve capacity of blood. Does a similar parallelism occur in quinine glycogenolysis? In the following table, table 4 are given data bearing on this point.

From table 4, it is to be seen that instead of a fall in reserve capacity, it either remained unchanged or more often actually rose. Only once in the series did the reserve capacity fall and this (no. 101) occurred simultaneously with an unusual rise in sugar. In all other instances there did not occur a significant fall, but on the other hand there appeared a rather striking rise in reserve capacity, with remarkable frequency of occurrence. Here, then, we have an agency capable of producing glycogenolysis intermediated by nerves and presumably the same nerves as are affected by epinephrine, without an accompanying fall in alkaline reserve capacity. Since in all instances of quinine hypergly-

⁶ Tatum, A. L., Jour. Pharm. and Exp. Therap., 1921, xvii, 395.

TABLE 4

The effect of quinine on blood sugar concentration and alkaline reserve capacity of whole blood

ANIMAL NUMBER	WEIGHT	TIME	BLOOD SUGAR	ALKA- LINE RE- SERVE*	NOTES
	<i>kgm.</i>	<i>1922</i>	<i>mgm./cc.</i>	<i>cc.</i>	
96a	1.7	January 24, 11:00 11:45	1.03	0.65	170 mgm. quinine HCl intramuscularly.
		1:15	1.41	0.86	
		2:30		0.76	
96b	1.8	January 25, 10:30 11:45	1.26	0.68	200 mgm. quinine HCl intramuscularly
		1:45	2.51	0.80	
97a	1.5	January 26, 9:30 10:00	1.13	0.86	200 mgm. quinine HCl intramuscularly
		12:00	2.51	0.94	
97b†	2.2	January 26, 3:00 3:50	1.03	0.81	220 mgm. quinine HCl intramuscularly
		5:50	1:08	0.81	
98a‡	2.2	January 27, 2:00 2:15	1.08	0.69	220 mgm. quinine HCl intramuscularly
		3:15		0.74	
		4:15	1.13	0.76	
99a‡	2.0	January 30, 11:30 11:45	1.00	0.75	220 mgm. quinine HCl intramuscularly
		1:45	0.90	0.80	
100a	2.2	February 1, 2:15 3:15	1.41	0.72 0.74	220 mgm. quinine HCl intramuscularly
		4:35	2.26	0.72	
100b	1.7	February 2, 2:30 3:00	0.94	0.76	200 mgm. quinine HCl intramuscularly
		5:00	1.08	0.78	

TABLE 4—*Concluded*

ANIMAL NUMBER	WEIGHT	TIME	BLOOD SUGAR	ALKA- LINE RE- SERVE*	NOTES
	<i>kgm.</i>	<i>1922</i>	<i>mgm./cc.</i>	<i>cc.</i>	
101	1.7	February 3, 2:00 2:30 4:30	1.13 2.83	0.79 0.74	200 mgm. quinine HCl intramuscularly
106a	2.0	February 21, 2:30 3:00 5:00	1.41 2.05	0.67 0.75	500 mgm. in 1 per cent by stomach
106b§	1.6	February 22, 10:25 10:35 11:25 12:45	 1.61 1.88	 0.47 0.76 0.80	500 mgm. in 1 per cent by stomach
107a	2.8	February 24, 2:15 2:30 3:50 5:15	 No Change	0.75 0.74 0.79	500 mgm. in 1 per cent by stomach
107b	3.0	February 25, 10:00 10:30 2:00	 No Change	0.74 0.77	750 mgm. in 1 per cent by stomach
108a	2.6	February 28, 10:30 11:00	1.10	0.84	250 mgm. subcutan- eously in 1 per cent solution
108b	2.0	March 1, 3:15 3:50 6:00	1.03 1.74	0.79 0.76	500 mgm. in 1 per cent by stomach

* Uncorrected values.

† Animal had been subjected some weeks previously to right splanchnotomy and left adrenalectomy.

‡ Double splanchnotomy performed some weeks previously.

§ Animal found dead three hours after last specimen of blood was drawn.

cemia there occurred some degree of hyperpnoea, and in the last series (table 4) the blood in no instance was venous or cyanotic in appearance, cardiac inefficiency was eliminated, and also depression of the respiratory center is shown to be quite improbable. As both acidosis and alkalosis cannot well coexist we are still more strongly convinced that glycogenolysis occurs as a specific type of cellular activity independent, to considerable extent, of other simultaneous cellular functions. Acid production, then, is to be considered a separate result of epinephrine action, while conservation of alkalies a separate result of quinine action, each capable of occurring simultaneously with glycogenolysis. Epinephrine may be considered as exerting a specific dynamic action on metabolism, having as one end result a degree of acidosis. Quinine on the other hand, depresses particularly protein metabolism leading to a degree of alkalosis.

In view of the observations recorded in this paper epinephrine secretion from the adrenal glands cannot consistently be held to be the *sine qua non* of quinine hyperglycemia since in only one manifestation (glycogenolysis) do they coincide, while in regard to acid-base balance they are diametrically opposite. This leads us to reiterate our view that epinephrine produces, as one manifestation of activity, glycogenolysis,—among others, acid production, bearing no demonstrable causal connection to glycogenolysis. We might otherwise draw the conclusion that quinine produces hyperglycemia because it causes a diminution of acidity! We are forced to believe, on the basis of available facts, that glycogenolysis is a common manifestation of both quinine and epinephrine, while one incidentally decreases, the other increases acidity. If epinephrine be the intermediate agency in quinine action, its activity as a physiological secretion differs from its activity as a drug. If epinephrine is not the intermediate agency, then we are forced to concede such a rôle to some other secretion of the adrenal glands or to some nervous manifestation.

Both epinephrine and quinine cause a concentration of blood, but in neither case can the concentration factor account on the one hand for a rise in sugar with a fall of reserve, or on the other hand, rise in sugar with rise of reserve, for in neither case, further-

more, is the concentration of a sufficient grade to account for the changes in sugar.

The hypoglycemia under quinine is easily accounted for on the basis of absence of extrinsic lytic nervous impulses coupled with a diminished capacity of intrinsic glycogenolysis.

SUMMARY

1. Quinine properly administered is a glycogenolytic agent.
2. Quinine hyperglycemia is the result of a central nervous system disturbance which leads by way of the splanchnic nerves and the normally innervated adrenal glands to lysis of glycogen.
3. In the absence of adrenal innervation quinine produces in most instances hypoglycemia, most likely by virtue of a peripheral depression of glycogenolysis.
4. Quinine produces in most instances examined a rise in alkaline reserve capacity of whole blood along with hyperglycemia in normals and hypoglycemia in animals with denervated adrenal glands.
5. Neither quinine nor epinephrine hyperglycemia can, on the basis of available evidences, be considered dependent upon acidosis.

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THE IMPORTANCE OF THE ADRENAL GLANDS IN THE ACTION OF PILOCARPINE, PHYSO- STIGMINE AND STRYCHNINE

CHARLES W. EDMUNDS

From the Pharmacological Laboratory of the University of Michigan

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In a recent paper (1) in which I discussed the point of attack of certain drugs acting at the periphery I paid particular attention to pilocarpine and I pointed out that there was no one single tissue which this alkaloid could be said to attack to the exclusion of others. There was evidence that it acted in certain parts of the body upon muscle substance proper (retractor penis muscle, ileo-colic sphincter); upon parasympathetic myoneural junctions in still others (iris); and again upon parasympathetic nerve endings (bronchial muscle). And finally that it was said to act in still other localities upon the terminations of the true sympathetic fibres (uterus). To these effects might perhaps have been added an action upon sympathetic ganglion cells a localization of pilocarpine effects which has been described by Dale and Laidlaw (2).

The action of the drug is seen therefore to be an exceedingly complex one and it was in an effort to simplify the matter that the experiments to be described later were carried out.

Of special interest and importance in this connection is the work of Cushny (3) which showed that the alkaloid acted upon the cat's uterus in situ in the same manner as does epinephrine and hypogastric nerve stimulation thus demonstrating an action connected with the sympathetics. Its contractor effect is antagonized by ergotoxine and both contractor and inhibitory effects are nullified by atropine, in this latter respect differing from epinephrine and hypogastric stimulation the effects of which are not changed by atropine. On account of these relationships it was

suggested that both pilocarpine and atropine act upon some structure connected with the sympathetic innervation to the uterus.

This paper published in 1910 was opposed to the view which had been commonly held regarding the effect of pilocarpine upon the uterus and which was described and pictured by Cushny in his earlier paper (4) published in 1906. Here pilocarpine is described as always causing contractions of the uterus and as being antagonistic to the inhibitory effects produced by hypogastric stimulation.

This question as to the point of attack of pilocarpine is of considerable interest and importance affecting as it does also atropine. It would indicate that both may act upon the terminations of the sympathetic mechanism and thus furnish an example of such an effect the only one in the body other than that of the sweat glands. It seemed therefore that a further study of the subject was justified.

A close study of Cushny's results as described in his 1910 paper suggested that they might perhaps be explained by an indirect effect of pilocarpine upon the uterus—that this alkaloid might act upon the adrenal glands stimulating them to an increased outpouring of epinephrine and that it was this substance which was responsible for the sympathetic effects which were seen after the injection of pilocarpine. Such an explanation would fit all the effects which are described in the paper including its similarity to hypogastric stimulation and its relationship to ergotoxine. This possible relationship to epinephrine was suggested by Dale and Laidlaw (2) and they carried out an extensive series of experiments looking toward this explanation of the question. They confirmed the findings of Cushny but found that sometimes they encountered a reverse condition in that pilocarpine would induce contractions in a uterus which in turn was inhibited by epinephrine. In other words the similarity of pilocarpine to sympathetic effects was subject to exceptions. As regards the effects of increasing the output of the adrenals they showed that in an eviscerated cat stimulation of the splanchnics not only raised the blood pressure but it also relaxed the uterus. They then showed that the hydrastinine group of alkaloids in the absence of the

adrenals no longer inhibited the non-pregnant uterus in situ. Finally coming direct to pilocarpine it was found that blood taken from the suprarenal vein after pilocarpine had a more powerful inhibitory effect upon the cat's isolated uterus than had blood obtained before pilocarpine had been given. In four experiments they found that the output of the epinephrine was increased by pilocarpine; in one experiment it was doubled while in another it seemed to be even more than doubled. As will be seen the evidence presented by Dale and Laidlaw of the relation of pilocarpine to increased epinephrine output is apparently clear but as is also seen it is not directly connected experimentally to the results reported by Cushny. This also seems to be the general view as shown by the fact that it is still commonly held that pilocarpine acts upon the sympathetic nerve endings in the uterus.

A factor of importance which is rather disturbing in this connection is that previous to the paper by Dale and Laidlaw other workers had failed to demonstrate any increase in epinephrine output whatsoever under the influence of pilocarpine. Dale and Laidlaw discuss briefly the work of Ehrmann, Tschobok-saroff, and Elliott all of whom reported negative results. The whole literature of the relation of pilocarpine to epinephrine output has been reviewed very fully recently by Stewart and Rogoff (5) and so need not be taken up in detail again by the present writer. The work of Dale and Laidlaw is discussed and Stewart and Rogoff point out that the amount of epinephrine which they (Dale and Laidlaw) reported as being found in the blood of the one cat which was accurately assayed was about twenty times as much as they themselves had found to be the average normal amount for cats. Also that if the amount had been doubled by pilocarpine the normal amount in this animal must have been ten times the average amount which had been found in the Stewart and Rogoff experiments.

The results obtained by Stewart and Rogoff however confirm the earlier reports in that they found that pilocarpine produced either no increase in the epinephrine output or only a very small one certainly one not comparable to the increase which is pro-

duced by physostigmine and by strychnine. In only one experiment was the amount increased to double that present in the blood before the pilocarpine had been administered but in this case the original quantity found was considerably below the average normal found in cats. In another animal was an increase probably present while in a third cat additional evidence of an action by pilocarpine was found in a moderate depletion of the epinephrine store in the adrenal glands with intact nerve supply.

The whole evidence therefore of the relation of pilocarpine to epinephrine output seems to point to the fact that the effects produced are relatively small. In certain cats the amount of epinephrine in the blood is doubled while in others no very clear increase can be made out. This situation would explain the apparently contradictory results reported by Cushny in his two papers and also the statements made by Dale and Laidlaw that they had found the inhibitory phenomenon to be an uncertain affair. On the face of it though it does not look as if the very trifling increase in epinephrine output could have been responsible for the inhibition of the uterus following pilocarpine especially when it would be opposed by the direct motor action of the pilocarpine upon the organ which is so easily demonstrated when the organ is isolated from the body and immersed in Ringer's solution. It seemed therefore worth while to examine the matter further using the direct method of giving pilocarpine both with the adrenals intact and also after they have been removed.

The cats employed were anaesthetized with chloretone, 0.3 gram per kilogram, dissolved in a little alcohol, diluted with water and given by the stomach tube. After arterial, venous and tracheal cannulae had been inserted the abdomen was opened in the median line. In certain experiments the adrenal glands were removed at once while in others they were removed at a later stage. Two silk threads were inserted in one of the uterine horns and the animal was then immersed in a saline bath maintained during the course of the experiment at a temperature of 37°C. In a number of the experiments the second horn of the uterus was removed and placed in cold Ringer's solution to be used later as an isolated structure to check against the results

obtained in the intact animal. The silk threads in the uterus were now attached to the two extremities of the recording apparatus and this in turn to the writing lever which was arranged to record the movements of the uterus in the usual manner. In passing it might be pointed out that the adjustment of the levers sometimes required repeated trials to ascertain the proper tension to bring out the best results from the individual organ. In general it was found that a minimum tension served the purpose best; the uteri unless they were large fleshy organs seemed incapable of withstanding any considerable tension. After the proper adjustments had been made an injection of epinephrine (1 cc. of a 1:25,000 solution) was given with the twofold object of testing the adjustment of the apparatus and of ascertaining the character of reaction brought out by sympathetic stimulation. It may be said that in every animal employed epinephrine injection was followed by inhibition of the uterus. It is true that many of the cats were fairly young weighing from 1800 grams to 2400 grams and the uteri were rather small but some were animals which had very recently been pregnant and had large and very vascular organs but they all responded alike with inhibition both in situ and also when used as isolated organs.

After recovery from the epinephrine 2 mgm. of pilocarpine nitrate were given intravenously and this was followed immediately by the great slowing of the heart and fall of blood pressure, characteristic of pilocarpine. A little later or about forty-five seconds after the alkaloid had been given there followed in some of the animals a marked relaxation of the uterus as described by Cushny. A rather noticeable feature of the reaction of the uterus was the unusually long latent period. This is clearly seen in Tracing No. I and is also plainly discernable in the tracings reproduced in Cushny's second paper.

The animal from which the tracing shown in figure 1 was taken was removed from the saline bath and both its adrenals excised and it was then replaced in the bath and all adjustments were made as before. The pilocarpine was still active as shown by the low blood pressure and slow heart. The uterus at this time was showing a slow undulating movement of contraction and



FIG. 1

FIG. 1. CAT. BLOOD PRESSURE (BELOW); UTERUS TRACING ABOVE

Lever moves up during relaxation of uterus. Effect of injection of 1 mg. pilocarpine on cat's uterus when adrenal glands are intact. Time record is in seconds.

FIG. 2

FIG. 2. SAME CAT AS IN FIGURE 1, EXCEPT THAT THE ADRENAL GLANDS HAVE BEEN REMOVED

Same arrangement of levers as in figure 1. Tracing shows effect of pilocarpine on the uterus of a cat after adrenalectomy.

relaxation. Another injection of pilocarpine (1 mgm.) was now given and this time there was no sustained stage of inhibition but a great increase in the strength of the movements of the uterus as is well shown in the tracing given in figure 2. There was thus a marked difference in the reaction of the uterus before and after removal of the adrenals—before, there was complete relaxation while after there was a marked increase in the strength of the spontaneous movements, an increase which was well sustained. No other explanation can be given for the change in the reaction of the organ than the presence or absence of the adrenals. The other half of this uterus which was removed before the experiment proper began was tested by the perfusion method and it gave the normal response to the two alkaloids, marked contraction and increased tone to pilocarpine and complete relaxation to epinephrine.

In other animals the adrenals were removed before the first injection of pilocarpine was given and here the alkaloid called forth strong contractions. This reaction however would not be conclusive evidence either for or against the theory of an intermediate action by epinephrine unless the results were obtainable in a very large series of cats inasmuch as Dale and Laidlaw have pointed out that the inhibitory effect of pilocarpine upon the uterus in situ is an inconstant affair and that they had encountered instances in which, even with the adrenals intact, pilocarpine had called forth contractions. And this inconstancy in reaction is of course to be expected as the result obtained in any given case is the sum of the direct motor effect of pilocarpine and of the inhibition produced by epinephrine. The motor effect is of course always present but the epinephrine increase is more uncertain as has been mentioned.

Another alkaloid which has the same general action upon the uterus as has pilocarpine is physostigmine and at the same time it has been shown that it too is capable of increasing the epinephrine output as has also strychnine. Physostigmine has been examined by Kurdinowski and by Cushny both of whom report strong contractions of the uterus following its use and Cushny says also that in no case has he seen inhibitory effects such as he

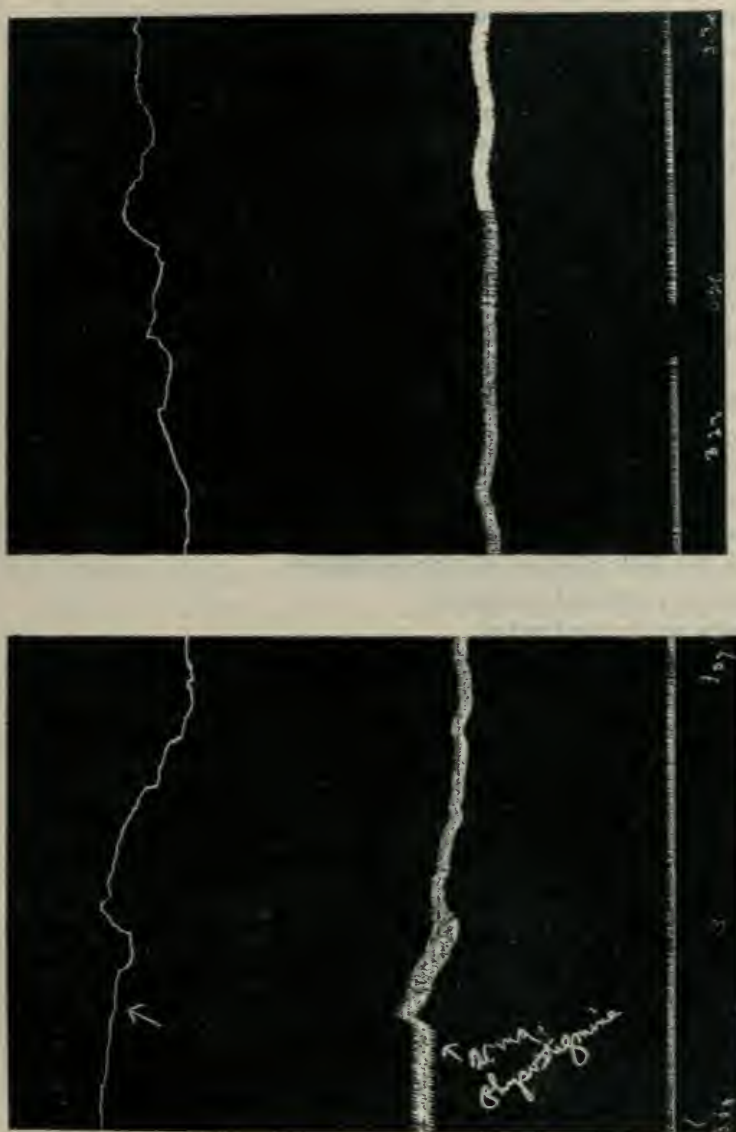
has described as following pilocarpine administration. In this connection it must be remembered that the direct motor effect of physostigmine upon muscular structures is much more marked than is the effect produced by pilocarpine and it is certainly possible these motor effects might easily offset completely any inhibition which might otherwise be present due to a possible increase in epinephrine.

In regard to this latter point there is the recent important contribution of Stewart and Rogoff (6) in which they report a great increase in epinephrine output under the influence of physostigmine. This increase which may equal ten or fifteen times the normal does not appear immediately, there being even a slight decrease during the first few minutes, the largest increase being found from twenty to thirty minutes after the alkaloid is injected. It will thus be seen that the effects which are usually described as being due to physostigmine are the immediate direct results of the alkaloid, that is, the direct motor action uncomplicated by any later increase in epinephrine. As to whether this increase in epinephrine could overcome the strong motor effects of physostigmine was therefore investigated using the same methods as were described under the section devoted to pilocarpine.

As was to be expected not all the cats reacted alike there being the two opposing forces, motor and inhibitory and it depended upon the dose of physostigmine and possibly upon the condition of the adrenals as to which effect predominated. A typical protocol may be given and the accompanying tracing is shown in figure 3.

December 1, 1921. Cat, 1800 grams; 0.7 gram chloretone. Cannulae inserted. Uterus adjusted and tested with epinephrine and ergot.

3.01 2 mgm. Physostigmine injected. Heart slowed, blood pressure fell, marked increase in tone of uterus (see tracing fig. 3). Intestines show marked increase in peristalsis. In place of being pink they become white with larger vessels outlined in bright red. During next 15 minutes conditions remain much the same with very strong gastric and intestinal peristalsis, long sections of intestine being absolutely white except for



3b

3a

FIG. 3. CAT WITH ADRENALS INTACT

Same arrangement of levers as in figures 1 and 2. Tracing 3a shows the primary effect of physostigmine in causing contraction of the uterus. Tracing 3b, continuation of 3a after an interval of 14 minutes. Tracing shows the gradual relaxation of the uterus which began about twenty minutes after the physostigmine had been given.

the largest blood vessels. Uterus remains fairly quiet without active contraction but with the increased tone mentioned. This tone began to relax very gradually twelve minutes after the physostigmine had been injected.

- 3.22 Distinct relaxation of uterus with organ returning to approximately normal conditions except that it was more active than at the beginning of the experiment. Organ remained in this same general condition during the next twenty minutes. Intestines have become quiet, relaxed and pink.
- 3.27 Intestines pink, quiet.
- 3.32 Intestines same. Occasional peristalsis in large intestine. Heart very slow.
- 3.35 Entire length of intestine pink, quiet and relaxed.
- 3.40-4.00 Both adrenals removed. Animal replaced in bath and recording apparatus readjusted.
- 4.05 2 mgm. physostigmine injected. Uterus which had regained its tone remained fairly quiet during next thirty-five minutes. There was no relaxation of the organ such as had appeared after the first injection of physostigmine.
- 4.06 Very active intestinal peristalsis.
- 4.08 Intestines white, contracted.
- 4.10 Same.
- 4.18 Peristalsis lessened but stronger than normal. Heart very slow.
- 4.30 Intestines paler than normal. Moderately active peristalsis.
- 4.35 Intestines constricted, pale, peristaltic bands.
- 4.40 Experiment terminated.

This experiment was very striking. In the early part the muscular effects of the physostigmine as shown by the uterus and intestine were very marked but about twenty minutes after the alkaloid had been given a change came over the picture, the uterus gradually relaxed and the intestines altered their appearance completely. Instead of being tightly contracted and white they became quiet, pink and completely relaxed.

These inhibitory effects could easily be explained by an increased outpouring of epinephrine produced by the physostigmine because in the latter part of the experiment after the adrenals had been removed inhibition was entirely absent, both uterus and intestine exhibiting increase tone until the end of the observations.

In not all animals were the results so clear cut as they were in the example just outlined. For instance in another cat the motor symptoms due to the alkaloid were much greater as shown by twitching of the voluntary muscles which phenomenon was absent in the previous cat. In this animal which had recently been pregnant there were marked spontaneous contractions of the uterus and these were increased in number and strength by the physostigmine. After about twenty minutes the conditions returned to approximately normal but there was never the total inhibition seen in the previous case described. The intestinal peristalsis also lessened to a considerable degree but here too the inhibition was not complete there being at intervals of every three or four minutes a strong contraction appearing at different parts of the tube. It was evident therefore in this animal that any possible increase in epinephrine due to the physostigmine was not sufficient to overcome completely the stimulant action of the physostigmine upon the muscular structures.

As strychnine also increases the output of epinephrine (an action to which reference has been made), at this point in the experiment 1 mg. of strychnine was injected intravenously—an amount sufficient to increase the reflexes in the animal. The effect so far as the uterus and intestines are concerned is best shown by brief extracts from the protocol, extracts taken immediately preceding and following the strychnine injection.

- 3.42 Strong contractions involving most of intestine. Uterus fairly active.
- 3.45 Strong contractions of intestine.
- 3.48 Intestines less active but occasional strong contractions.
- 3.50 1 mgm. strychnine intravenously.
- 4.00 Intestine pale—quiet—no peristalsis. Uterus active.
- 4.05 No peristalsis.
- 4.15 No intestinal peristalsis but intestines are fairly pale. Uterus distinctly less active.

In this experiment too the effects were unmistakable. A uterus spontaneously active has its movements increased in number and in strength by physostigmine and the intestine fares

likewise. The movements in both organs continue but after some time they decrease somewhat but never disappear. Following a small dose of strychnine some decrease in activity is seen in the uterus but the effects are much plainer in the intestines. After about ten minutes they become absolutely quiet but they never gained the markedly dilated condition which was seen to follow the physostigmine injection in the animal previously described. This difference was doubtless due to the direct effect of the physostigmine. As to the cause of the change produced by strychnine recourse must be had once more to the work of Stewart and Rogoff (7) upon the effect of strychnine upon epinephrine output. They found that even small doses of strychnine, doses which might be considered to be well within the therapeutic limits, would increase very markedly the output of epinephrine even to many times the normal amount found for the animal. This increase comes on within a few minutes and it may last for over an hour. There would seem little doubt but that the change which came over the intestine in the animal described and to a lesser degree the uterus was due to increased epinephrine output produced by the strychnine and that the final picture in both organs was the sum of spontaneous activity, of physostigmine stimulation, and of epinephrine inhibition.

Control experiments to those described above were carried out by administering physostigmine to cats after adrenalectomy in order to ascertain whether the effects of the alkaloid were prolonged to a considerable degree, that is, as to whether the motor effects continued for a longer time in animals without adrenals than when the adrenals are intact. This was found to be the case. For instance in one cat the uterus showed markedly increased tone and contraction almost an hour after the alkaloid had been injected while at the same time the protocol shows that the intestine was still active there being "marked peristalsis over long sections of the intestine." In like manner strychnine administered in doses of from 0.5 to 1 mgm. to cats with intact adrenals was followed in about fifteen minutes by considerable relaxation of the uterus.

DISCUSSION

All my results tend to show that the adrenal glands if they are stimulated to increased activity by certain alkaloids may affect very considerably the effects of those alkaloids. The action of pilocarpine producing inhibition of the cat's uterus when the organ is in its normal relation is doubtless an example of such an action and pilocarpine therefore in this instance does not act upon the sympathetic nerve terminations. The motor effect of physostigmine upon both uterus and intestine is also greatly modified at times by an increased output of epinephrine an increase which reaches its height at from twenty to thirty minutes after the alkaloid has been given. This increase in epinephrine may even at times completely remove all the motor manifestations of the physostigmine. It is a question whether such an action may not have considerable significance from the clinical standpoint. Physostigmine has quite commonly been utilized in the treatment of intestinal atony and too often with disappointing results. Its administration to patients is frequently followed by signs of an action upon the intestine as shown by the passage of flatus, but too often such an action is quite transient and no further evidence of an effect is apparent. May it not very well be that the direct motor action of the physostigmine in these cases is entirely nullified by the effect of its action upon the adrenals? Certainly in the animals referred to such a train of events followed.

Another very interesting observation which was made (but which was not new) was the greater sensitiveness of the intestines over the uterus to the epinephrine secreted. As was shown in the protocols it happened frequently that the intestines would be rendered perfectly quiet and relaxed while the uterus was still undergoing contractions. This was true especially in those cases in which the uterus was active before the physostigmine was injected. On the other hand if the uterus was quiet before the physostigmine was given it was not infrequently restored to this state sometime after the alkaloid had been administered.

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AN INVESTIGATION INTO THE CHEMOTHERAPY OF THE ACRIDINE DYES IN EXPERIMENTAL TUBERCULOSIS¹

MAURICE I. SMITH

From the Division of Pharmacology, Hygienic Laboratory, United States Public Health Service, Washington, D. C.

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INTRODUCTORY

The possible applicability of the acridine dyes in chemotherapeutic research was first shown by the basic experiments of Browning and his associates. Following Ehrlich's observation of the curative properties of diamino-methyl-acridinium chloride when injected into mice infected with trypanosomes, Browning and Gilmour (1) showed that the dyes of the acridine group, in common with many other dyes, possess considerable specificity against certain groups of bacteria, a property which is not exhibited by the heavy metal disinfectants, e.g., mercury, silver or gold. It was further pointed out that the bactericidal action of the compounds of the acridine group against many forms of bacteria was unaffected, or actually enhanced by the presence of serum in the medium, a remarkable property, which as far as is known, is not shared by any other chemical agent.

In 1917, Browning, Kenneway, Gulbransen and Thornton (2) extended their earlier observations, and summed up the following important advantages for diamino-methyl-acridinium chloride (acriflavine): (a) High antiseptic and bactericidal action, unchanged or augmented by the presence of serum; (b) relative freedom from inhibitory effects on phagocytosis; and (c) relative freedom from irritating effects on mammalian tissues. For the above reasons they recommended the use of this

¹ Approved for publication by the Surgeon General.

substance for local application in the treatment of bacterial infection in wounds. Later Browning and Gulbransen (3) further showed that the serum of an animal treated with diaminoacridine-sulphate (proflavine) is endowed with antiseptic and bactericidal properties against *Staphylococcus aureus* and *B. coli* for several hours following the injection. The animals were not harmed by the treatment, and the authors suggest the possibility of the use of this substance in chemotherapeutic research. The slowly progressive bactericidal action of these substances in serum would further suggest the possibility of more active substances being elaborated in the animal organism following their administration.

Gay and Morrison (4) fully corroborated the above findings, and further applied diamino-methyl-acridinium chloride (acriflavine) in the treatment of experimental streptococcic empyema. The results of their experiments, however, were negative.

Franz (5) reported experiments showing that acriflavine possesses considerable bactericidal action against *B. diphtheriae*. Neufeld, Schiemann and Baumgarten (6) claim to have obtained favorable results with acridine orange, a related substance, in the treatment of chicken cholera.

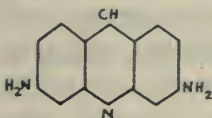
There appears to have been but little work done with the compounds of the acridine group in experimental tuberculosis. Lewis (7) studying the inhibiting action of numerous dyes on the tubercle bacillus found acridine orange to exhibit a high degree of efficiency in restraining the growth of the bovine tubercle bacillus on a medium of glycerin broth. It is further of interest to note that this substance showed an antiseptic action against the tubercle bacillus 250 times as great as that against the typhoid bacillus, indicating perhaps a certain degree of specificity against the acid-fast type of organisms.

As this work was nearing completion, a paper by Petroff (8) appeared giving the results of his experiments with acriflavine and proflavine in experimental tuberculosis, which were for the most part negative. It may be stated at once that the results of the experiments described herein fully corroborate the findings of Petroff, in so far as treatment of tuberculous animals with acridine dyes did not seem to influence favorably the course of

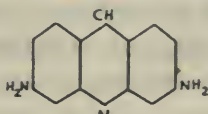
the infection. Because of the wider scope of this work, however, it seems desirable to record these experiments. It may further be added that Petroff apparently employed a highly virulent type of infection, and he resorted to the subcutaneous administration of the compounds used for treatment. In my experience the subcutaneous administration of the acridine dyes caused marked local reactions, which must make the matter of absorption uncertain. In these experiments the intravenous route was therefore used for the administration of the compounds. A relatively mild type of tuberculous infection was used with a view of making it possible to detect even slight advantages that might accrue from the treatment, if such were the case.

ACRIDINE COMPOUNDS USED IN THIS WORK

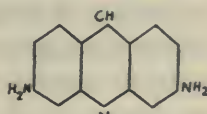
The compounds employed in these experiments are derivatives of the acridine base. Acriflavine, or diamino-methyl-acridinium chloride was first prepared by Benda in 1912 (9), and named trypanflavine by Ehrlich owing to its trypanocidal action. Proflavine, which is somewhat less toxic, is diamino-acridine-sulphate. These two compounds were purchased in the open market. The dimethyl derivative of diamino-methyl-acridinium chloride, or briefly acridinium yellow, and acridine orange, which is the methylated acridine base, as well as the silver compounds of acriflavine and of proflavine were kindly prepared by Dr. J. M. Johnson, organic chemist of this laboratory.



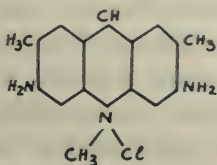
ACRIDINE BASE



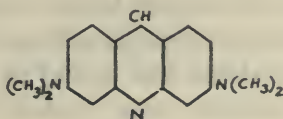
ACRIFLAVINE



PROFLAVINE



ACRIDINIUM YELLOW



ACRIDINE ORANGE

The silver compound of acriflavine was made by treating an alcoholic solution of the dye with an alcoholic solution of silver nitrate, whereupon the compound precipitated out. This was centrifuged, washed with alcohol, and dried in vacuum desiccator. The silver compound of proflavine was prepared in essentially the same manner, with the exception of aqueous instead of alcoholic solutions of the dye and reagent being used. The former compound contained 29.4 per cent silver, and the latter 11.3 per cent.

EXPERIMENTAL

1. The inhibiting action of the compounds on the growth of the tubercle bacillus

This was determined by inoculating flasks, containing glycerin broth and the compounds in solution in varying concentrations, with a culture of human tubercle bacilli, and incubating these at 38°C. The technic of these experiments was essentially the same as that employed in previous work (10). All the compounds under discussion were found to possess a very high degree of inhibiting action on the growth of the tubercle bacillus, and very nearly of the same extent. Thus acriflavine and proflavine and their silver derivatives caused complete inhibition in dilutions up to 1:200,000. Partial inhibition was observed with acriflavine in as high a dilution as 1:1,000,000. Acridinium yellow effected complete inhibition up to 1:500,000. The same concentration showed complete inhibition with acridine orange, and partial inhibition was evident in 1:1,000,000 dilution, which is quite in accord with the observations of Lewis (7). It would appear that the introduction of methyl groups, especially when attached to a N atom, increases the effectiveness of the compound against the tubercle bacillus, as was also pointed out by Browning and Gulbransen with regard to trypanosomes (3). It may be of interest to note that in subeffective concentrations these compounds frequently gave a more rapid and more luxuriant growth than was found in the control flasks, which would seem to indicate that in low concentrations these substances actually accelerate the growth of the tubercle bacillus.

2. Effect on the pathogenicity of the tubercle bacillus

This was determined by the same method as in a previous publication already referred to (10). The washed tubercle bacilli after exposure for forty-eight hours at body temperature to the action of the compounds in definite concentrations were injected intraperitoneally into guinea-pigs, which were carefully examined at autopsy, and the findings frequently confirmed by microscopic examination of the tissues. The results of these experiments are given somewhat in detail in table 1, which show, as is frequently the case, that none of the compounds examined has a bactericidal or a demonstrably attenuating effect on the tubercle bacillus, even though they are capable of restraining its growth to a marked degree.

3. Is the serum of acridine treated animals inhibiting or bactericidal for the tubercle bacillus?

Browning and Gulbransen (3) showed that proflavine injected intravenously into rabbits confers bactericidal properties on the blood of such animals against *Staphylococcus aureus* and *B. coli* lasting several hours following the injection. In view of the relatively high inhibiting action of the acridine compounds on the tubercle bacillus in vitro, as disclosed by the foregoing experiments, it was thought it might be possible to demonstrate inhibiting properties in sera of treated animals against this bacillus. This possibility appeared more probable from the observations of the above authors to the effect that the bactericidal action of acridine compounds in serum develops slowly and progressively, suggesting the possibility of formation of more active substances in the animal organism.

Rabbits were injected with proflavine 1:400 in physiological salt solution in one of the ear veins. After a variable length of time about 40 cc. of blood were withdrawn from the heart with a sterile syringe and immediately transferred into a sterile centrifuge tube. On the following day this was centrifugalized. Ten cubic centimeters of the serum was transferred into a sterile flask of 100 cc. capacity and inoculated with a loopful of human

TABLE 1
Effect of acridine compounds on the pathogenicity of the tubercle bacillus. After forty-eight-hour exposure at 38°C. injected into guinea-pigs intraperitoneally

CONCENTRATION	DAYS	DIED OR CHLOROFORMED	Spleen	Liver	Omentum	Peritoneum	Lungs	Glands
Acriflavine								
1:100,000	42	Died	Enlarged, nodular	Caseous necrosis	Miliary tubercles	Bloody exudate	Tuberculous pneumonia Miliary tubercles Few miliary tubercles	Enlarged
1:10,000	63	Chloroformed	Not involved	Not involved				Enlarged caseous
1:1000	77	Chloroformed	Slightly enlarged, miliary tubercles	Few miliary tubercles	Miliary tubercles			
1:100	108	Chloroformed	Enlarged, nodular	Caseous necrosis	Nodular			Enlarged
Silver compound of acriflavine								
1:100,000	79	Chloroformed	Enlarged, nodular	Miliary tubercles		Few nodules		Enlarged
1:10,000	79	Chloroformed	Numerous miliary tubercles	Few tubercles		Few nodules		Enlarged
1:1000	77	Died	Much enlarged, caseous	Caseous necrosis	Nodular		Miliary tubercles	Caseous

Proflavine.

1:100,000	61	Chloro- formed	Enlarged, nodular	Numerous mili- ary tubercles	Miliary tuber- cles	Many nod- ules	Miliary tu- bercles	Enlarged
1:10,000	61	Chloro- formed	Enlarged, miliary tubercles	Few tubercles	Miliary tuber- cles			Enlarged
1:1000	57	Died	Enlarged, miliary tubercles	Numerous mili- ary tubercles	Miliary tu- bercles		Few tubercles	Enlarged
1:100	61	Died	Enlarged, caseous necrosis	Caseous necrosis	Nodular		Few tubercles	Caseous

Silver compound of proflavine

1:100,000	82	Chloro- formed	Slightly enlarged, nodular.	Few miliary tubercles	Miliary tuber- cles			Enlarged
1:10,000	83	Chloro- formed	Slightly enlarged, few nodules	Free	Miliary tuber- cles			Enlarged
1:1000	83	Chloro- formed	Free	Free	Free	Free	Free	Free
1:100	15	Died		Few tubercles		Few tu- bercles		Enlarged

Acridine orange

1:10,000	82	Chloro- formed	Epitheleoid pro- liferation	Free	Epitheleoid pro- liferation			
1:1000	82	Chloro- formed	Epitheleoid pro- liferation	Free	Epitheleoid pro- liferation			
1:100	82	Chloro- formed	Enlarged, nodular	Caseous necro- sis	Miliary tuber- cles			Enlarged

TABLE 2
Shows absence of inhibiting or tuberculocidal properties in serum of rabbits treated with proflavine intravenously

EXPERIMENT NUMBER	WEIGHT kilos	PRO-FLAVINE INJECTED mgm. per kilo	BLOOD WITHDRAWN HOURS AFTER INJECTION	GROWTH OF TUBERCLE BACILLUS ON SERUM	PERIOD OF OBSERVATION OF TEST GUINEA PIG days	DIED OR CHLOROFORMED	AUTOPSY FINDINGS
1	2.5	25	2	Slight, but distinct	30	Died	Bloody exudate in peritoneal cavity. Spleen and glands enlarged. Many miliary tubercles in spleen and liver
2	2.1	50	4	Dried out	67	Chloroformed	Numerous miliary tubercles of spleen and liver, former much enlarged. Miliary tubercles in lungs glands and omentum.
3	3.2	25	24	Slight, but distinct	67	Chloroformed	Tuberculous nodules in spleen, omentum and peritoneum. Glands enlarged.
4	2.5	25	48	Slight growth (contaminated)	3	Died	No autopsy
5		0	Control	Contaminated	2	Died	General peritonitis

tubercle bacilli on the surface of the medium. After a month of incubation at 38°C. the flasks were examined for growth. To test its tuberculocidal action 5 cc. of the serum were placed in a small sterile centrifuge tube, three drops of a heavy emulsion (10 mgm. to 1 cc.) of human tubercle bacilli (H 37) added, and incubated at 38°C. for forty-eight hours. At the end of this time the tubercle bacilli were centrifugalized, washed with sterile physiological salt solution, and finally taken up in 1 cc. sterile salt solution and injected intraperitoneally into guinea-pigs. At autopsy the extent of tuberculous involvement was noted. The results of these experiments are given in table 2. The experiments are few; they clearly indicate, nevertheless, that the serum of an animal taken two hours or longer after treatment with proflavine is not capable of inhibiting the growth of the tubercle bacillus nor of altering its pathogenicity to a marked degree. If it is possible to produce such an effect on the serum by means of proflavine it must be of very brief duration, and therefore of doubtful practical value.

4. Therapeutic value of acridine compounds in experimental tuberculosis

a. Experiments on guinea-pigs. The results of the foregoing experiments made it appear doubtful if the dyes under consideration could exert a decided influence on the tuberculous process in experimental animals. Nevertheless, owing to the marked inhibiting action of these substances on the growth of the tubercle bacillus in vitro it seemed desirable to examine their effects in experimentally infected animals. To this end a series of 45 guinea-pigs were inoculated intraperitoneally with 0.1 mgm. tubercle bacilli, human type (H 37) in 1 cc. physiological salt solution. This, it was found previously, produces a rather mild infection, generalizing slowly, and running a relatively long course. This series of animals was divided into three groups. Group 1 consisted of 17 guinea-pigs which served as controls. Group 2 contained the same number of pigs, kept under the same conditions as those of the preceding group, but received weekly

intravenous injections of 5 to 10 mgm. per kilo of 0.5 per cent solution acriflavine. In group 3 there were 11 guinea-pigs receiving weekly intravenous injections of 10 mgm. per kilo of the silver compound of acriflavine.² The treatments had to be discontinued after 6 injections, as there are only six superficial veins in the guinea-pig that are available, and it has not been possible to use any one vein more than once. Subcutaneous injection was attempted, but the local reactions were so severe, characterized by ulceration, sloughing and gangrene, that it was given up.

The infecting dose of tubercle bacilli was intentionally small, as stated above, and the disease ran a relatively long course, so that after 220 days of observation some of the animals in each group were still living, which were at this time chloroformed and autopsied. The animals surviving the 220-day period were 4, 9 and 4 in the respective groups, or 23, 53 and 36 per cent respectively. This apparently speaks in favor of the treatment. Actually it is doubtful if this signified anything more than the normal variation, especially when viewed in the light of further analysis.

Upon comparing the average length of life of the animals that died during the observation period of 220 days it was found that the controls lived to an average of 149 days, the animals of group 2 had an average of 161 days of life, while those of group 3 only lived 98 days. Or if groups 2 and 3 be combined it appears that 15 treated animals lived an average of 131 days as against 13 controls living to an average of 149 days. Chart 1 shows graphically the death curve of the controls and treated animals, and illustrates more vividly in another way the figures just quoted. The continuous line represents the death curve of the 13 controls, while the broken line, which is more steep, shows a higher mortality rate of the 15 treated animals (8 of group 2 and 7 of group 3).

² Preliminary experiments showed that guinea-pigs safely tolerate in single doses up to 20 mgm. per kilo of acriflavine, and 10 mgm. per kilo of the silver compound of acriflavine.

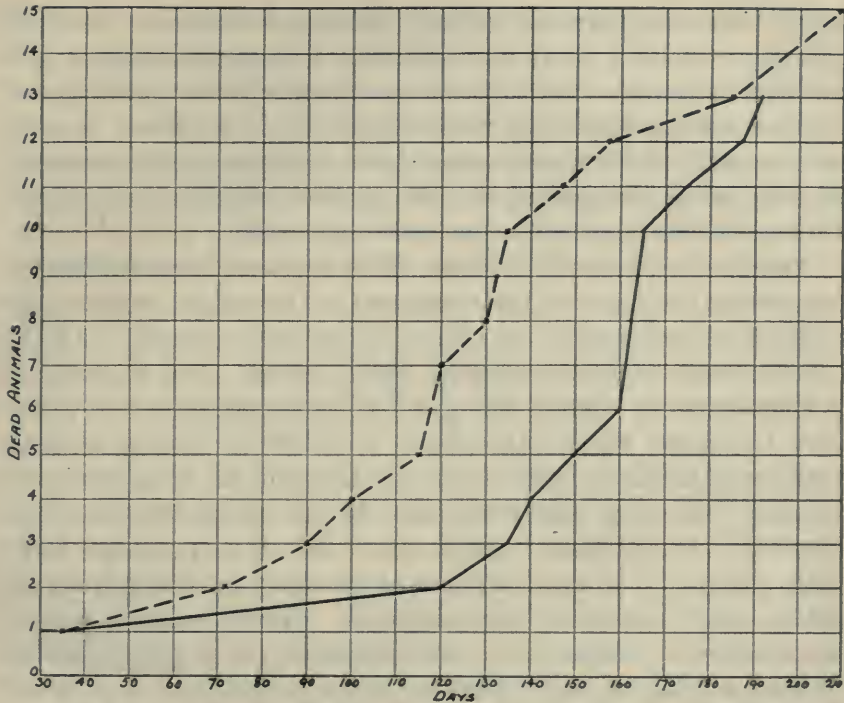


CHART I. DEATH RATE OF GUINEA-PIGS INOCULATED WITH *BACILLUS TUBERCULOSIS*

Solid line-thirteen controls. Broken line-fifteen treated animals, eight with acriflavine, and seven with the silver compound of acriflavine.

TABLE 3

Extent of tuberculous involvement found at necropsy in the 3 groups of guinea-pigs

ANIMALS SHOWING	GROUP 1 (17 CONTROLS)	GROUP 2 (17)	GROUP 3 (11)
	per cent	per cent	per cent
No lesions.....	12	12	27
Localized tuberculosis.....	18	12	18
Moderate generalization.....	35	53	36
Extensive generalization with caseous necrosis.....	35	23	19

All the animals were carefully autopsied, and the extent of tuberculous involvement noted. Examination of table 3 will

show that there are no striking differences between the three groups. Group 3, it is true, presents a somewhat higher percentage of animals showing no lesions, and a lower percentage of animals with extensive involvement, which is, however, entirely accounted for by the somewhat higher death rate of the animals of this group, as pointed out above, thus affording less opportunity for the tuberculous process to generalize.

Another criterion that might be taken into consideration in appraising the value of the treatment is the weight curve of the controls as compared with that of the treated animals. All the animals were carefully weighed once a week. But in order to get comparative figures which are not misleading it was necessary to choose the weight charts of relatively young growing males which showed a fair degree of tuberculous involvement at autopsy, and of as nearly the same extent in the two groups as possible. A composite weight curve was thus prepared for 6 male controls, 3 of which showed at necropsy moderate generalization, and 3 extensive generalization. For comparison a similar composite weight curve was prepared for 8 treated males (6 with acriflavine and 2 with the silver compound), 4 of which showed at necropsy moderate generalization and 4 extensive generalization. The initial weight of all these animals just before inoculation was very nearly 400 grams. Chart 2 shows the comparative average weight curves of the two groups of animals. It appears that the animals of both groups were gaining steadily for about 15 weeks following inoculation, and then declined rather slowly but progressively. The two curves practically run parallel, and there is but little evidence in favor of one or the other group.

Summing up the results of the experiments on guinea-pigs it may be safely concluded that there is no definite evidence of the acridine compounds affecting tuberculous infection in any appreciable manner.

b. Experiments on rabbits. The guinea-pig, as is well known, is highly susceptible to tuberculous infection, and the process tends to generalize relatively rapidly in this animal even if only small doses of tubercle bacilli are used for inoculation. It seemed

desirable therefore to extend these studies to a more chronic type of tuberculous infection such as follows the intratesticular inoculation in rabbits with a human strain of tubercle bacilli (11). In this type of experimental infection the tuberculous process while progressive, yet remains localized for the most part within the inoculated tissues, and may be examined at definite intervals, thus affording an easy means for the evaluation of the chemotherapeutic agent under consideration. Two groups of

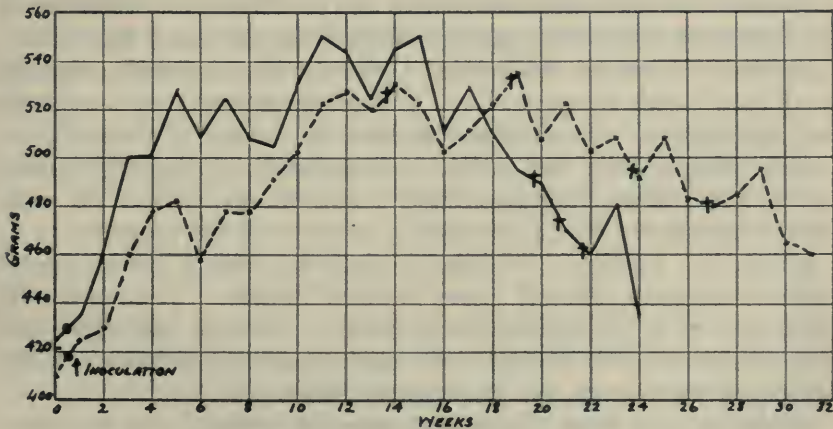


CHART 2. AVERAGE WEIGHT CURVE OF CONTROL AND TREATED ANIMALS

Solid line represents the average weight curve of six control male guinea-pigs, three of which showed at autopsy moderate, and three extensive generalization of the tuberculous process. Broken line shows the average weight curve of eight treated males (six with acriflavine, and two with the silver compound), four of which showed moderate and four extensive tuberculosis. At 0.01 mgm. human tubercle bacilli injected intraperitoneally. Sign † indicates death of an animal.

rabbits, five in each, were used in these experiments. The animals of the first group were treated with proflavine 1:500 in physiological salt solution intravenously, and the animals of the second group with the silver compound of acriflavine 1:500 in distilled water by the same route. The experiments are detailed below.

Group 1. Proflavine

Rabbit 1. 2.4 kilos. April 19, inoculated intratesticular with 5 mgm. human strain tubercle bacilli (H 37). May 5, animal developed

hard nodule at upper pole of the inoculated testicle. May 10, 17, 24 and June 2, injected 20 mgm. per kilo proflavine intravenously. The tuberculous lesion remained the same. June 9, the animal was chloroformed. At autopsy there was extensive caseation of the epididymis. On histologic examination the spleen also revealed a few young tubercles.

Rabbit 2. 2.8 kilos. Right testicle inoculated April 19. May 5, a small nodule at the lower pole was found on examination. Proflavine in doses of 20 mgm. per kilo were injected on May 10, 17 and 24 and June 2. By June 16, the inoculated testicle had become nodular throughout. Upon examination both the epididymis and the body of the testicle showed diffuse miliary tuberculosis with some caseation.

Rabbit 4. 1.8 kilos. Inoculated April 19. May 5, testicle presented small hard nodule at upper pole. Proflavine treatment given as in the last experiment. Examination on June 30 showed a distinctly enlarged testicle, hard and nodular throughout. At autopsy diffuse miliary tuberculosis with considerable caseation was found in the epididymis and testicle, and a few miliary tubercles in the lungs.

Rabbit 10. 2.2 kilos. Inoculated April 19. May 5, the inoculated testicle was much enlarged, tense, firm and nodular. Treatment with proflavine as in the previous experiments. June 16, the organ was much enlarged, and studded with large caseating tubercles. A few miliary tubercles were also found in the lungs.

Rabbit 11. 2.6 kilos. Inoculated April 19. May 5, the inoculated organ was slightly enlarged, and several small nodules were found at the upper pole. Three injections of 20 mgm. per kilo each were given at weekly intervals. June 16, the organ remained unchanged in size, and the small nodules had coalesced, forming a large hard nodule in the upper pole. At autopsy the epididymis showed diffuse miliary tuberculosis.

Group 2. Silver compound of acriflavine

Rabbit 8. 2.3 kilos. Inoculated April 19. May 5, three small nodules were found in the inoculated organ. On May 10, 17 and 24, 10 mgm. per kilo were injected intravenously. By June 9 the testicle had become moderately enlarged and nodular throughout. Autopsy showed numerous miliary tubercles in the body and epididymis.

Rabbit 12. 2.5 kilos. Inoculated April 19. No lesions were apparent at the time treatment was begun. Weekly injections during the month of May were given, four in all, of 10 mgm. per kilo each.

Examination June 9 revealed a much enlarged, firm and nodular testicle. At autopsy the lungs presented a few miliary tubercles besides the extensive diffuse tuberculosis and caseation of the testicle and epididymis.

Rabbit 13. 1.8 kilos. Inoculated April 19. By May 5 there had developed a small hard nodule at the upper pole. Treatment was instituted as in the last experiment. June 9, the lesion appeared unchanged. Autopsy showed caseous necrosis of the epididymis.

Rabbit 14. 2.2 kilos. Inoculated April 19. No lesion was apparent on examination on May 5. Treatment during May as in the last experiment. June 9, the inoculated testicle appeared slightly enlarged and nodular. Histologic examination showed numerous conglomerate tubercles with caseating centers.

Rabbit 18. 2.5 kilos. Inoculated April 19. A hard nodule was found at the upper pole on May 5. Treatment with the silver compound as in the previous experiments. June 9, the testicle was moderately enlarged and nodular. Autopsy July 18 revealed extensive caseous necrosis of the epididymis.

It is obvious from the foregoing experiments that neither proflavine nor the silver compound of acriflavine exerts the slightest influence on the tuberculous process. The lesions developed in these animals much in the same manner as they do in untreated animals. Sections were made in each case and carefully examined microscopically for any evidence of healing such as fibrosis, cicatrization or encapsulation, but none was ever found.

CONCLUSIONS

The acridine derivatives, acriflavine, proflavine, and their silver compounds, acridinium yellow, and acridine orange possess a high degree of inhibition on the growth of the tubercle bacillus in vitro.

These substances do not alter the pathogenicity of the tubercle bacillus when exposed to their action at 38°C. for forty-eight hours.

Experiments made to determine whether the serum of animals treated with proflavine would show inhibiting or bactericidal properties for the tubercle bacillus yielded negative results.

Proflavine, acriflavine and the silver compound of the latter were applied in the treatment of experimental tuberculosis in the guinea-pig and in the rabbit. No effect on the tuberculous process was noted.

I wish to express my indebtedness to Professor Carl Voegtlin for suggesting this problem, and to Dr. J. M. Johnson for preparing some of the compounds used in this work.

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TOXICITY AND ACTIONS OF THE NORMAL BUTYLAMINS

P. J. HANZLIK

From the Departments of Pharmacology, Leland Stanford Junior University, San Francisco, and Western Reserve University, Cleveland

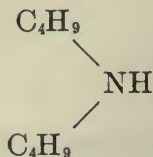
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Of the two forms of butylamins, the normal butylamins are generally regarded as more active pharmacologically than the isobutylamins. However, information about the normal butylamins is fragmentary and conjectural, while the isobutylamins have been shown to be sympathicomimetic, cardiac depressant and convulsant poisons (S. Fränkel, 1). Normal butylamin and some other related amins occur in dark cod liver oil and, according to Kobert (2), stimulate glandular secretions. In view of the therapeutic importance of cod liver oil, the butylamins merit some attention. Therefore, a survey of the toxicity and pharmacological actions of the normal butylamins was made in connection with a study of some phenylenediamins previously reported (3). The present paper summarizes the results on mammals obtained with the normal butylamins, which were found to possess rather definite and unexpected actions.

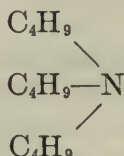
I. DESCRIPTION OF THE COMPOUNDS STUDIED

Monobutylamin. This is a colorless, volatile fluid with a penetrating, nauseating ammoniacal odor; boiling point, 76°C. Its formula is $C_4H_9 \cdot NH_2$.

Dibutylamin. When freshly prepared, dibutylamin is colorless, slightly oily and more viscid and less volatile, therefore, less penetrating than the mono compound, but still has a disagreeable, ammoniacal odor; boiling point, 161°C. On standing it becomes yellowish. Its chemical formula is



Tributylamin. This compound is a colorless, oily fluid when fresh, but changes to a brownish oil on standing. It is the least volatile of the series, less penetrating, and the peculiar nauseating ammoniacal odor is least marked, though distinctly recognizable. Its formula is



and boiling point, 215°C.

All of the butylamins are basic in nature; relatively insoluble in water but freely soluble in such lipoid solvents as alcohol, kerosene, turpentine, chloroform and liquid petrolatum. When treated with acids they form salts which are readily soluble in water. Monobutylamin fumes, when exposed to air and in the presence of volatile acids, behaving in this respect like ammonia. This is less pronounced with dibutylamin and absent with tributylamin.

II. FATAL DOSAGE AND COMPARATIVE TOXICITY

The fatal dosage of the butylamins was determined on 87 white rats, injecting the compounds subcutaneously as such, i.e., in basic form, and in the form of hydrochlorides in aqueous solutions, usually of 1 per cent strength. In addition, dibutylamin was administered hypodermically and gastrically to 12 rabbits. The gastric administrations were made through a small rubber catheter attached to a glass bulb, using a little water for washing down the compound. A fatal dose was one that caused death on the same or following day of administration. The results obtained are presented in the accompanying table, which indicates the distribution of fatalities in correspond-

ing ranges of dosage for each compound, and, therefore, the variations that were encountered. The compounds may be discussed individually, reserving a description of the symptoms for the next section.

Monobutylamin. The 27 white rats that were injected with this compound responded variably. The mortality was 75 per cent with a dosage of 2 to 2.5 cc. per kilo; $66\frac{2}{3}$ per cent with 1.5 cc., and 30 per cent with 0.6 to 1 cc. per kilo. Irrespective of variations, the minimal fatal dose appears to be somewhat less than 0.6 cc. per kilo.

Dibutylamin. The results on 38 white rats were also variable; 3.5 to 5 cc. per kilo were fatal to 100 per cent or all rats injected with this dosage. Sixty-five per cent died from a dosage of 2 to 3 cc. per kilo; 0.5 to 1 cc. per kilo killed 69 per cent, and all that were injected survived 0.1 to 0.45 cc. Therefore, the minimal fatal dosage, irrespective of variations, lies between 0.45 cc. and 0.5 cc. or a mean of 0.47 cc. per kilo.

Dibutylamin was also injected hypodermically in 3 rabbits. Two rabbits which received 1 cc. and 2 cc. per kilo, respectively, died, and one rabbit, which received 0.5 cc. per kilo, survived. Accordingly, the minimal fatal dose for rabbits (hypodermically) would appear to be somewhat higher, i.e., a mean of 0.75 cc., than for white rats by the same method of administration. The compound was also administered gastrically to 9 rabbits. One hundred per cent of 6 animals that received 1 to 2 cc. per kilo died and the 3 animals that received 0.7 to 0.9 cc. per kilo survived. Therefore, the minimal fatal dose by stomach for rabbits is about 0.95 cc., which is higher (about 20 per cent) than that hypodermically (0.75 cc. per kilo), as would be expected. The results obtained with both hypodermic and gastric administrations indicate that rabbits are less susceptible than white rats to dibutylamin.

Tributylamin. Twenty-two rats were injected with this compound. Dosages that ranged from 1.5 to 2.5 cc. per kilo killed 100 per cent of 3 that were injected; 69 per cent of 13 rats died from a range of 0.5 to 1 cc. per kilo and none of the 6 rats died from a range of 0.3 to 0.4 cc. per kilo. According to this

the minimal fatal dose lies between 0.4 cc. and 0.5 cc., or a mean of 0.45 cc. per kilo.

Vapor toxicity. Monobutylamin is rather volatile; fumes when exposed to the air and the vapors attack the eyes, causing lachrymation. Dibutylamin is less volatile, but 2 cc. and 5 cc. quantities placed on cotton under a slightly raised bell jar of 5-liter capacity produced sufficiently concentrated vapors at ordinary room temperature to kill 5 white rats rather promptly, death occurring at the end of three-fourths, one and one-half, twenty, twenty-three and twenty-four minutes, respectively, after exposure. One rat survived fifteen days and on the fifteenth day died of pulmonary edema. Using the flowmeter-bubbler method in the same way as with dimethylparaphenylenediamin previously described (3), the lowest fatal concentration of the vapors for 15 white rats exposed to concentrations ranging from 1:121,000 to 1:50,000 was found to be about 1:75,000. The symptoms with the higher concentrations occurred promptly and were exactly the same as those from hypodermic injection of the compound, and, therefore, were due to alveolar absorption of the dibutylamin. Unfortunately, enough monobutylamin was not on hand for carrying out quantitative experiments on vapor toxicity. However, since it is volatile, the vapors are very likely toxic. On the other hand, the tributylamin is oily and relatively non-volatile. Saturated vapors from this compound under a bell jar were not fatal to white rats, and therefore, quantitative experiments were not necessary.

Comparative toxicity. The results on fatal dosage by hypodermic injection in white rats just presented indicate that the toxicity of the butylamins increases with increase in the number of butyl groups. That is, monobutylamin is the least and tributylamin the most toxic, dibutylamin occupying an intermediate position. This is illustrated chiefly by the distribution of fatalities with corresponding ranges of dosage of the different compounds in the accompanying table. The percentage mortality was greater in corresponding ranges of dosage as the complexity of the compounds increased, i.e., from the mono- to the tributylamin, and the percentage of survivals was greater in the reverse

direction, i.e., towards monobutylamin. The same tendency, though somewhat less definitely, is shown by the minimal fatal dosage, namely, in descending order as follows: monobutylamin <0.6 cc., dibutylamin 0.47 cc. and tributylamin 0.45 cc. per kilo. These results confirm the statement of S. Fränkel (1) that the physiological action of the butylamins increases with the size of the molecule.

TABLE 1
Toxicity of butylamins for white rats (injected hypodermically)

RANGE OF DOSAGE ADMINISTERED	NUMBER OF ANIMALS USED	MORTALITY
Monobutylamin		
<i>cc. per kilo</i>		<i>per cent</i>
2.0 to 2.5	8	75
1.5	3	66 $\frac{2}{3}$
0.6 to 1.0	16	30
Dibutylamin		
3.5 to 50	8	100
2.0 to 3.0	8	65
1.5	1	0
0.5 to 1.0	13	69
0.1 to 0.45	8	0
Tributylamin		
2.0 to 2.5	2	100
1.5	1	100
0.5 to 1.0	13	69
0.3 to 0.4	6	0

The results with vapor toxicity are too incomplete for purposes of comparison, and in any case would be modified by the volatility of the compounds. Tributylamin, being least volatile, would be the least toxic, although hypodermically it was found to be the most toxic.

III. SYMPTOMS

Locally, the butylamins are non-irritating to the skin and only mildly irritating to the mucosae. Neither rubefaction nor vesication was produced when the compounds were applied liberally in basic form to human skin.

Systemically, they produce rather marked symptoms, which are similar to those produced by other alkylamins and the ammonium ion on injection. The observations were made on the same white rats and rabbits that were used for determination of the fatal dosage. Both species behaved the same, and the three compounds produced similar symptoms. Hypodermic injections caused restlessness, increased reflex excitability, increased pulse rate and respiration during the excitant stage, dyspnea, convulsions of the medullary type at first, later spinal, followed by depression, slowing of the pulse and respiration, marked cyanosis of mucosae and tongue, coma and death. In rabbits, convulsions were usually preceded by fascicular twitching of facial, orbital and cervical musculature. The fatal doses and time of death were variable. One cubic centimeter per kilo subcutaneously was fatal, as a rule, to rats in about six minutes after administration. Gastric administration to rabbits gave the same results as hypodermic administration.

At autopsy, the lungs were found collapsed; all viscera were congested and cyanotic. As a rule, the heart was dilated and intestinal peristalsis fairly active. The blood was invariably rather dark.

IV. CIRCULATION

Observations were made on 8 dogs, which were previously morphinized, curarized and, in some experiments, atropinized, in order to determine the mechanism of action on the circulation independently of convulsions and other changes. Blood pressure was recorded from the carotid artery by a damped mercury manometer in the usual way. At the same time changes in cardiac volume were recorded by a tambour attached to a ball cardioplethysmograph (Y. Henderson's) and kidney volume was recorded from an oncometer. All records were made simultaneously on a slow moving kymograph. Injections of the compounds were made intravenously by means of a graduated syringe and through a metal stopcock cannula in the saphenous vein. There were all told 3 injections of monobutylamin, 3 of tributylamin and 14 of dibutylamin in different animals. The effects

with the different butylamins were qualitatively the same, differing somewhat quantitatively. Figure 1 illustrates typical circulatory effects of dibutylamin in an atropinized dog, and also of the mono- and tributylamins in an untreated animal.

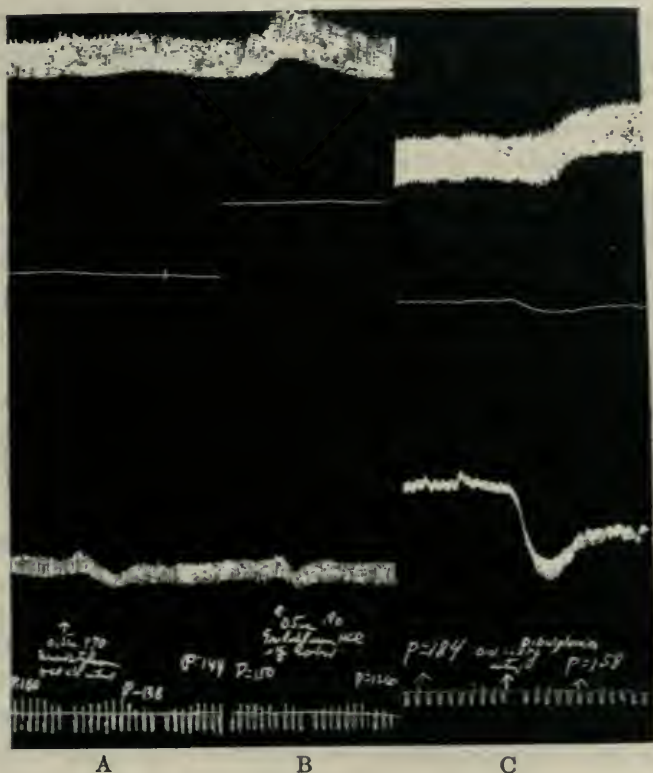


FIG. 1. EFFECTS OF NORMAL BUTYLAMINS ON THE CIRCULATION IN DOGS

A. Monobutylamin hydrochloride, 0.005 cc. per kilo intravenously in dog 21 (11 kilos).

B. Tributylamin hydrochloride, 0.005 cc. per kilo intravenously in dog 21 (11 kilos).

C. Dibutylamin, (0.01 cc. per kilo of base intravenously in dog 14 (15.6 kilos), whose vagi were paralyzed by previous injection of 0.1 cc. of atropin 1:10,000 per kilo intravenously. No recovery at end of ten minutes after injection of dibutylamin when another drug was injected.

All the compounds show depression of the circulation. P = pulse rate. Time, each stroke = five seconds.

The effects of small and moderate doses were characterized by a prompt fall of blood pressure, which was more or less proportional to the dosage, slowing of the pulse rate, increased cardiac volume and diminution in kidney volume, all of which indicates that the effects were of cardiac origin. The different functions recovered gradually though rarely to their previous conditions. In other words, the circulation remained permanently impaired. This was true of small doses ranging from 0.00085 cc. to 0.005 cc. per kilo. With these and somewhat higher doses (up to about 0.01 cc. per kilo) the duration of action varied from one minute to three minutes. Much larger doses, i.e., 0.04 cc. to 0.3 cc. per kilo intravenously accentuated the effects of the smaller doses; the blood pressure reached the zero level and the heart stopped in diastole. Such measures as the intravenous injection of epinephrin, Tr. digitalis and caffein and direct cardiac massage together with injection of warm 0.9 per cent sodium chloride usually resuscitated the circulation temporarily, but eventually the blood pressure returned to the zero level permanently. This was quite different with the phenylenediamins, which usually injured the circulation more severely (3).

The results were too variable to give an accurate idea of the comparative toxicity of the butylamins intravenously. Using the minimal dosage necessary to cause a fall of blood pressure as an index of toxicity the results showed that about 0.005 cc. per kilo was necessary for monobutylamin, 0.001 cc. per kilo for dibutylamin and 0.002 cc. per kilo or tributylamin. For definite and unmistakable effects the following doses were found to be necessary; monobutylamin 0.01 cc., dibutylamin 0.0017 cc., and tributylamin 0.005 cc. per kilo. There is, therefore, no definite relation between dosage and size of molecule, dibutylamin requiring the lowest effective and definite doses. However, monobutylamin appears to be the least toxic and tributylamin occupies an intermediate position.

In two dogs whose vagi were paralyzed to electrical stimulation by injection of atropin (0.1 cc. of 1:10,000 per kilo intravenously), the injection of dibutylamin and monobutylamin produced exactly the same effects as in unatropinized animals.

Therefore, the fall of blood pressure and slowing of the pulse produced by these compounds must be due to direct depression of cardiac musculature independently of the vagus mechanism. This agrees with the general statement of S. Fränkel (1) about the higher alkyl amines, namely, that they are cardiac depressants. However, the effects of small doses of the butylamins on smooth muscle of excised organs are just the opposite. The butylamins resemble histamin in its depressant action on the circulation and stimulant action on smooth muscle.

V. SMOOTH MUSCLE OF EXCISED ORGANS

The following organs were studied; intestine, uterus (pregnant and non-pregnant); and aorta of rabbit; guinea pig's bladder; pig's ureter; excised eye (pupil), stomach and cloaca of frog. The usual technique was employed. That is, longitudinal strips of the hollow viscera were suitably suspended in warm aerated Tyrode's solution (150 cc.) and the contractions were recorded on a slow moving kymograph. Aorta was used in the form of rings. The excised eyes of frogs were placed in small rubber rings and irrigated directly with the compounds or solutions of them. Frog's vessels were perfused with dibutylamin, using the L  wen-Trendelenburg technique. Figures 2, 3 and 4 illustrate typical responses of the intestinal and uterine musculatures to the different butylamins after preliminary treatment with nicotin and atropin and subsequent treatment with atropin, epinephrin and chelidonin for an analysis of their action. A great many tracings were obtained, most of which are too long or large for convenient reproduction. Therefore, the records here reproduced and a summary of the results must suffice.

The actions of the butylamins on smooth muscle of untreated intestine, uterus, bladder, ureter and stomach are characterized by a marked and sustained increase in tonus, with corresponding diminution or abolition of amplitude and a variable increase or no change in rate of contractions. These effects occur promptly and immediately after the application of the agents. This holds for concentrations from 1:75,000 to 1:3,000.

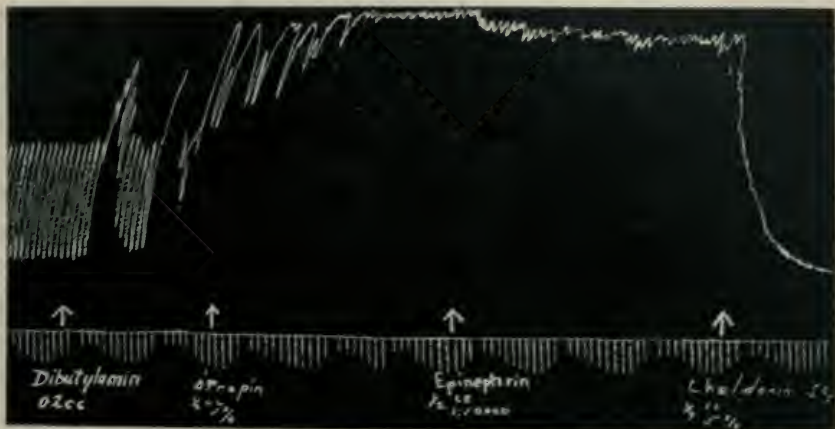


FIG. 3. EFFECT OF DIBUTYLAMIN (1:7500, END CONCENTRATION OF BASE) ON LONGITUDINAL STRIP OF RABBIT'S INTESTINE IN 150 CC. TYRODE'S SOLUTION AT 38°C.

Shows marked augmentation (especially tonus) of peristalsis unrelieved by atropin (applied end of six minutes) and epinephrin (applied end of thirteen minutes), but completely antagonized by chelidonin at end of sixteen minutes after application of dibutylamin. Time, each stroke = five seconds.

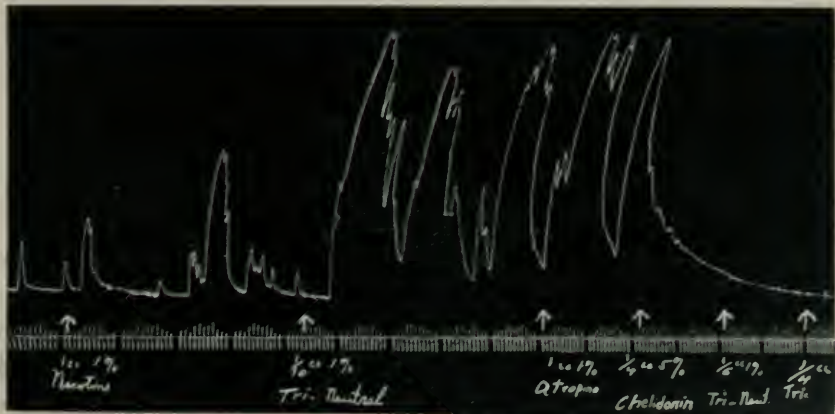


FIG. 4. EFFECT OF TRIBUTYLAMIN (1:15,000, END CONCENTRATION) HYDROCHLORIDE ON STRIP OF NICOTINIZED RABBIT'S PREGNANT UTERUS

Shows that augmentation is not antagonized by atropin, but completely relieved by chelidonin, with paralysis of the muscle, further applications of tributylamin remaining ineffective. 150 cc. of Tyrode's solution at 38°C. was used. "Tri" means tributylamin hydrochloride (practically neutral). Time, each stroke = five seconds.

tine. The augmentation was only partially relieved, or not at all, by treatment of intestine with atropin and epinephrin, and of uterus with atropin, but it was promptly and completely abolished by treatment with chelidonin, which acts by direct muscular depression independently of nerve connections. Therefore, the augmentation was due to stimulation of the muscle itself. A similar analysis gave similar results on ureter, bladder and stomach.

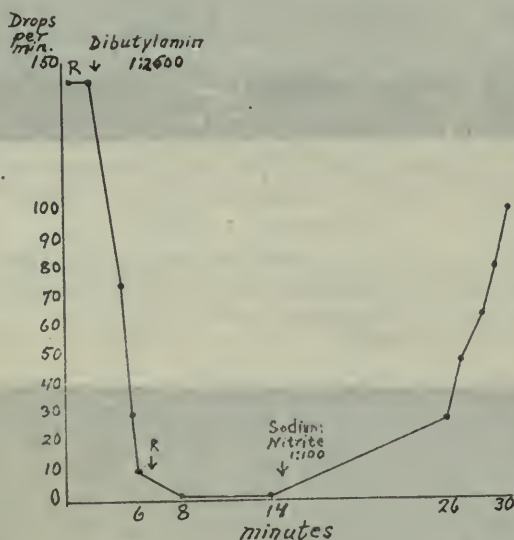


FIG. 5. EFFECT OF DIBUTYLAMIN (1:2500 BASE) ON PERFUSED VESSELS OF FROG'S EXTREMITIES

Shows vasoconstriction, unrelieved by Ringer's solution, but almost completely relieved by sodium nitrite. R = frog's Ringer's solution.

Rabbit's aorta showed a marked increase in tonus which was promptly relieved by chelidonin. The peripheral blood vessels of 5 frogs were perfused with concentrations ranging from 1:5000 to 1:700 of dibutylamin (neutral and basic). Constriction occurred with all concentrations that were tried, being most marked with the highest concentration that was used. The constriction was relieved better by sodium nitrite than by Ringer's solution alone. Figure 5 illustrates the action on frog's vessels.

The pupillary musculature of frog's eyes responded variably and imperfectly. Only the dibutylamin was tried. The four eyes that were used showed no effects with concentrations of 2 per cent of the base. Application of the base itself caused moderate relaxation of pupil from 2.5 to 3 mm. (diameter) in half an hour. The pupil failed to constrict in light and with 1 per cent solutions of pilocarpin and barium chloride. The pupil of one eye dilated from 3 to 5 mm. (diameter) in fifty minutes and remained permanently dilated despite repeated irrigations for two hours with 1 per cent pilocarpin and 10 per cent barium chloride, indicating that the pupillary musculature was paralyzed. Irrigations of four eyes in two rabbits with a 2 per cent solution of dibutylamin (base) caused moderate relaxation in two of them. The other eyes remained unchanged. There was no reaction of the dilated pupils to light and the dilatations were not antagonized by the application of 1 per cent pilocarpin and 1 per cent barium chloride. This, therefore, confirms the effects on excised eyes of frogs and the results are interpreted as a depression of the pupillary muscle directly by dibutylamin. However, high concentrations are necessary for this and the results were too inconsistent to attach much importance to them. Low concentrations of the compound were ineffective.

An attempt was made to estimate the comparative augmentor activity of the butylamins on smooth muscle of intestine and uterus and correlate this with the number of butyl groups in the compounds. Despite considerable variations in the responses of different strips from these organs, the summary indicated that the most marked tonus effects were produced by the mono- and dibutylamins and least by tributylamin. This was illustrated better by many other records, which cannot be reproduced for lack of space, than by those in Figures 2, 3 and 4, which are reproduced for other reasons. The changes in rate and amplitude of peristalsis were too variable to be used for this. The order of the butylamins in their activity on smooth muscle did not agree with the order of their activity as indicated by fatal dosage, but approached more that indicated by changes in blood pressure. The differences between the butylamins in their

physiological activity are not very large, and if to this are added the considerable variations that were encountered in different directions, it is obvious that accurate correlation of molecular weight or complexity of composition with physiological action is impossible, and generalization from the effects on a single function is misleading.

As compared with epinephrin, which is one of the most powerful augmentors for pregnant uterus, it was observed several times that the addition of monobutylamin and dibutylamin further markedly increased the tonus which had been previously raised by epinephrin. There is no doubt that these butylamins are superior tonus augmentors of smooth muscle in surviving organs.

VI. CONCLUSIONS

1. The normal butylamins are toxic and possess definite pharmacological actions.

2. The minimal fatal dosage per kilo by hypodermic injection in white rats was about as follows: <0.6 cc. for monobutylamin, 0.47 cc. for dibutylamin and 0.45 cc. for tributylamin. In rabbits the minimal fatal dose of dibutylamin hypodermically was 0.75 cc. per kilo and 0.95 cc. per kilo by stomach.

3. Monobutylamin and dibutylamin are sufficiently volatile to be toxic in vapor form, while tributylamin is relatively non-volatile and saturated vapors from it were non-toxic for white rats.

4. Locally, the normal butylamins are non-irritating to human skin and only mildly irritating to mucosae.

5. Systemically in white rats and rabbits, effective doses of all the normal butylamins cause increased reflex excitability, convulsions, increased pulse and respiratory rates during the excitant stage, dyspnea, marked cyanosis followed by depression slowing of the pulse and respiration, coma and death with large doses.

6. Intravenously in dogs, which were previously morphinized curarized and atropinized, all the butylamins caused a prompt fall of blood pressure, slowing of the pulse, increase in cardiac

volume and decrease in kidney volume, due to direct depression of cardiac musculature, independently of the vagus mechanism. As a rule, the circulation remained permanently impaired.

7. Smooth muscle of the following excised organs, uterus, intestine, ureter, stomach, aorta and perfused frog's vessels, was markedly stimulated by all the butylamins, as indicated by a marked increase in tonus, the rate and amplitude of contractions of peristaltic organs being variably affected or not at all. The pupils of excised frogs' and of intact rabbits' eyes gave variable and unimportant responses with liberal applications of dibutylamin.

8. The tonus augmentation was due to direct stimulation of smooth muscle independently of nerve connections, since it was not prevented and relieved by nicotin and atropin in intestine, stomach and uterus and by epinephrin in intestine. However, it was completely prevented and abolished in all organs by chelidonin, which depresses muscle exclusively of ganglia and nerve endings.

9. The results obtained with the butylamins on different functions were too variable, and in some cases too limited, to justify any generalization as to correlation of physiological activity with the number of butyl groups in the compounds.

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THE PHARMACOLOGICAL PROPERTIES OF SOME ISO-UREA DERIVATIVES

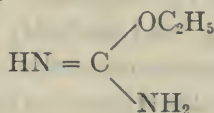
STEWART BASTERFIELD

*From the Laboratory of Pharmacology, University of Chicago and the Chemical
Laboratory, University of Saskatchewan, Saskatchewan, Canada*

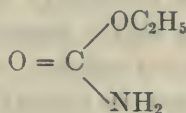
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Substituted ureas have been prepared in considerable number, and used with varying degrees of success as sedative and hypnotic drugs. Urea being a relatively innocuous product of metabolism has offered a convenient base on which to build a variety of synthetics of both the open-chain and cyclic ureide types. In view of the properties of such compounds it might be expected that alkyl iso-ureas and their derivatives would show similar physiological action. The great chemical reactivity of the iso-ureas and their ready conversion into ordinary urea derivatives suggests them as a useful starting point for synthetic work.

The iso-ureas closely resemble the urethanes in structure, as indicated by the formulas for ethyl-iso-urea (1) and ethyl urethane (2):



(1)



(2)

The iso-urea is simply imino-urethane, or the imino-ester of carbamic acid, and is a much stronger base than urethane. This fact would point to a less marked sedative action for the former, and possibly some stimulant action. Experiment showed that in moderate doses, the iso-urea had no appreciable depressant action, but though a mild degree of restlessness followed the administration of the compound it was not possible perhaps to interpret this definitely as stimulation.

The amidine grouping $\text{NH}_2 - \text{C}' = \text{NH}$ in the iso-ureas suggests also a relationship with the guanidines, a point worthy of note in view of certain muscular effects that were observed in rabbits during this study of the action of iso-urea derivatives.

The introduction of acyl groups into the iso-urea molecule, to diminish the basic properties, might be expected to uncover any sedative property inherently associated with the ether grouping $\equiv \text{C} \cdot \text{OC}_2\text{H}_5$, and it was with this idea in mind that a number of simple acyl-iso-ureas were prepared and tested pharmacologically. It may be stated here that only one compound, viz.: the carbethoxy derivative, O-ethyl-allophanic ester (ethoxy-allophanic ester), $\text{NH}:\text{C}(\text{OC}_2\text{H}_5) \cdot \text{NH} \cdot \text{COOC}_2\text{H}_5$ showed sufficient physiological action to warrant further investigation, and in this case, though the depressant action was relatively slight and transient, other unlooked for effects came to light which added interest to the whole problem and widened the field of investigation.

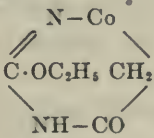
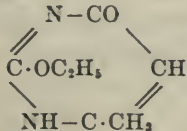
Certain acyl-iso-ureas have already been studied pharmacologically. Bayer and Company¹ have described the preparation of bromo-iso-valeryl-iso-ureas, and claimed for them valuable sedative properties. The chemistry of the acyl-iso-ureas was developed by Stieglitz (1) and his collaborators, and the method of preparation described in the Bayer patent is substantially the same as that previously worked out by Stieglitz. The sedative action of the Bayer compounds may, and probably is, due to a combination of effects, since bromo-compounds are notably depressant and the valeryl radicle is also reputed to have a similar influence.

EXPERIMENTAL

Most of the experiments were carried out with the rabbit as subject, though some preliminary tests were made with mice and dogs. In the case of the carbethoxy-iso-urea, the effect on the cat was also studied in one instance. Table 1 conveniently summarizes the experiments with compounds, other than the carbethoxy derivatives.

¹ Aust. Pat. 72,300.

TABLE 1

COMPOUND	ANIMAL	DOSE AND MODE OF ADMINISTRATION	RESULTS
Ethyl-iso-urea $\text{NH}_2\text{C}(\text{OC}_2\text{H}_5):\text{NH}$	Rabbit	0.5 gram per kilo in water; subcutaneous injection	Accelerated respiration; temperature fell 1.2°C. in two hours. Some restlessness
Acetyl-ethyl-iso-urea $\text{CH}_3\text{CO}\cdot\text{NH}\cdot\text{C}(\text{OC}_2\text{H}_5):\text{NH}$	Rabbit	0.5 gram per kilo in oil; subcutaneous injection	No appreciable effect
Benzoyl-ethyl-iso-urea $\text{C}_6\text{H}_5\text{CO}\cdot\text{NH}\cdot\text{C}(\text{OC}_2\text{H}_5):\text{NH}$	Mouse	50 mgm. in oil; subcutaneous injection (mouse weighed 20 grams)	Gradual depression; responded sluggishly to stimuli. Died
	Rabbit	0.5 gram per kilo in oil; subcutaneous injection. Repeated after 1 hour	No appreciable effect except a fall in temperature of 0.5°C.
	Dog	0.35–0.4 gram per kilo in suspension stomach tube	No effect
2-Ethoxy-barbituric acid 	Mouse	50 mgm. in water; subcutaneously	Well marked depression in half an hour; complete recovery in five hours
	Rabbit	0.5 gram per kilo in water as sodium salt; subcutaneously	No effect*
2-Ethoxy-4-methyl-uracil 	Dog	0.35 gram per kilo in suspension; stomach tube	No effect
	Rabbit	0.5 gram per kilo in water; subcutaneous injection	No effect

* The iso-urea salt of this acid was also tried without effect.

It will be noted that the only appreciable depressant effects were elicited with the benzoyl-iso-urea, and 2-ethoxy-barbituric acid, by means of heavy doses administered to mice. Larger animals were practically unaffected by moderate doses.

The fall of temperature obtained with ethyl-iso-urea itself is interesting, as a similar effect, but more pronounced, was obtained with the carbethoxy-compound. The rapid transformation of the iso-urea into normal urea in the body, probably accounts for the somewhat surprising absence of marked physiological action with such a strong base.

O-ETHYL-ALLOPHANIC ESTER

This derivative of ethyl-iso-urea (carbethoxy-ethyl-iso-urea) prepared for the first time in connection with this study is a weakly-basic, crystalline substance, readily soluble in alcohol, ether, hydrocarbons, and fatty oils, but only slightly soluble in water. It has a melting-point of 39°C .

The most marked physiological action was obtained when the freshly-prepared substance was administered in solution in olive oil, by subcutaneous or intramuscular injection. When administered by the mouth in capsules, the drug was much less potent, a fact probably explained by its rapid destruction by hydrolysis in the gastro-intestinal tract. It should also be noted that, as a base, the ester would dissolve in the acid gastric juice to form the hydrochloride, the latter substance then losing ethyl chloride (1), and becoming transformed into allophanic ester. This ester, in moderate doses, was found during the present study to have little if any depressant action.

The general affects produced in the rabbit by the injection in oil of 0.5 to 0.75 gram per kilo of the carbethoxy-iso-urea, were: first, a moderate depression of about 1 hour's duration; second, a rapid fall of body temperature during one to two hours, amounting, in some cases to as much as 3°C ., and third, an increased muscle tonus, particularly noticeable in the large muscles of the back and of the hind legs.

DEPRESSION

With reference to the first point, the degree of depression is admittedly a difficult quantity to determine, but a comparison may be drawn, perhaps, with well known hypnotics, such as

urethane, barbital, and luminal. The carbethoxy-iso-urea given by injection produced a greater and more rapid depression than urethane in equal doses given by the same method, but the effect was less persistent than that of urethane. However, when the latter was administered per rectum in aqueous solution, the depression developed as rapidly, and was as intense as with the carbethoxy-iso-urea, but was again more persistent. The rate of absorption seems to be an important factor in the comparison. The speed of absorption of the iso-urea derivative given subcutaneously in oil is surprising, the effects of the drug being evident in from five to ten minutes after injection.

Barbital and luminal (as sodium salts) given by subcutaneous injection in aqueous solution, in doses of 0.35 gram per kilo, produced depression which reached a maximum in two and one-half to three hours, and was far more profound and persistent than that induced by either the carbethoxy-iso-urea or the urethane.

EFFECT ON TEMPERATURE

The rectal temperature was found to fall rapidly in the first hour, the minimum being reached in one and one-half to two hours after administration of the drug. Recovery took place fairly quickly the temperature being normal again in four or five hours. As an example in one case using a dose of 0.75 gram per kilo the temperature of a normal rabbit fell from 38.6°C. to 35.6°C. in two hours. Two hours later the thermometer indicated 37.5°, and after a further interval of two hours it showed 38.2°.

Table 2 shows a series of observations for a typical case with some corresponding temperatures for a control animal. Controls never showed variations in temperature of more than 0.4°C.

Table 3 gives some additional data.

The rapid fall of temperature observed suggested that other causes than depressed metabolism and muscular inactivity might be operating, particularly since the increased muscle tonus would be expected to counteract to some extent the diminished heat production in other tissues. Comparisons as to rate of fall of

temperature, were accordingly made on the one hand, with urethane, barbital, and luminal in doses sufficiently large to cause

TABLE 2

*Effect of carbethoxy-ethyl-iso-urea on body temperature in rabbit.
Dose 0.5 gram per kilo. Mild depression*

SUBJECT		CONTROL	
Time	Temperature	Time	Temperature
<i>p.m.</i>	$^{\circ}\text{C.}$	<i>p.m.</i>	$^{\circ}\text{C.}$
3:20	38.7	3:20	39.0
3:40	38.7	3:50	39.2
4:00	37.8	(Animal active)	
4:15	36.8	4:20	39.0
4:30	36.4		
4:45	36.3	4:50	38.8
5:00	36.6		
5:15	37.0	5:20	38.8
5:30	37.3		
6:00	37.4		
7:30	38.6	7:30	39.0

TABLE 3

Comparative fall of temperature during one hour

SUBJECT	DATE	DOSE (TOTAL)	FALL OF TEMPERATURE IN 1 HOUR	REMARKS
	1922	gram	$^{\circ}\text{C.}$	
Rabbit A (1200 grams).....	April 23	0.75	1.3	After forty-eight hours fast
	May 1	0.70	1.0	
	May 3	0.70	1.9	
Rabbit B (1400 grams).....	April 28	0.70	0.9	After twenty hours fast. Total fall was 1.7° in one hour twenty minutes
	April 29	0.80	1.3	
	May 5	0.80	1.3	

marked and prolonged depression, and on the other, with acetanilide, a typical antipyretic.

The results with the hypnotics are most conveniently compared by means of the charts in figures 1 and 2. The curve for

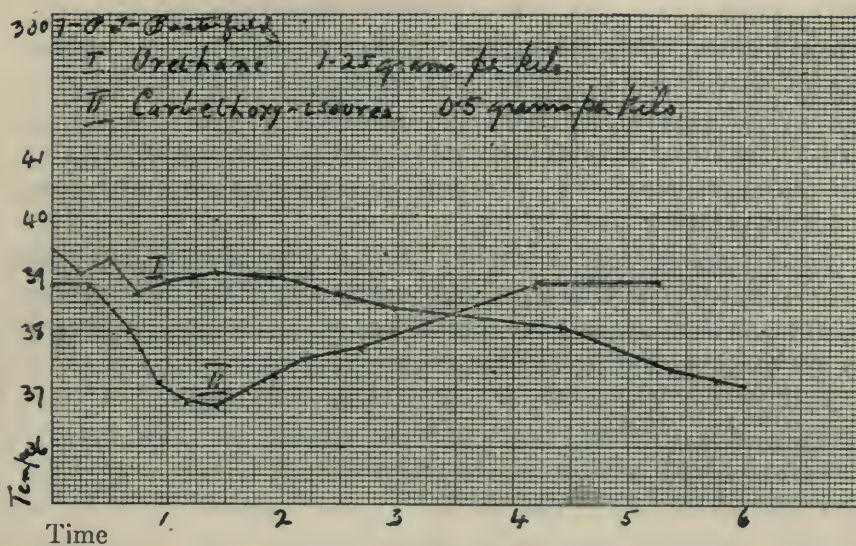


FIG. 1

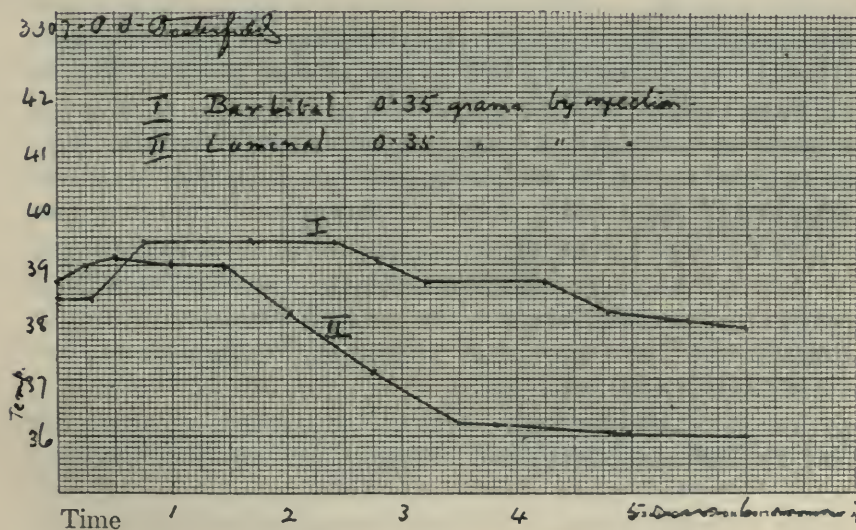


FIG. 2. EFFECT OF HYPNOTICS ON BODY TEMPERATURE OF RABBIT

the carbethoxy-iso-urea is of a different type from those for the other drugs. While with the urethane, barbital, and luminal the fall of temperature is slow and prolonged and seems to follow the depression and loss of activity, with the iso-urea compound, both the rate and extent of the fall seem to be out of proportion to the degree of depression, and point to an increased heat loss as the main factor in the situation.

The administration of acetanilide in a dose of 0.15 gram per kilo² produced, without noticeable depression, a fall of temperature of 1.2°C. in one and one-half hours, an effect more comparable with that of the carbethoxy-iso-urea.

In the cat, with a dose of 0.5 gram per kilo, the fall of temperature observed was slower and not so large as in the rabbit. During one hour a fall of 0.7°C. was obtained, in two hours 1.1°, and in five hours 1.7° (38.2 to 36.5°).

EFFECT OF MUSCLE TONUS

One of the most peculiar effects of the carbethoxy-iso-urea was the muscular rigidity that developed rapidly after the administration of the substance. The hind legs, in the rabbit, were gathered up considerably behind the normal position for the crouching animal, and the muscles were tense and resistant to attempts to straighten the limbs. In one case when the effect of the drug was at its maximum, the ears were held erect, not withstanding the obvious general depression. In another case, tremors developed when the temperature was at its lowest point, and recurred at short intervals until the temperature was returning to normal. Whether the tremors were due to an incipient tetanus, or were merely a shivering reaction due to low temperature is difficult to say.

In the cat, the muscle effect was not so marked, but there was much restlessness, crying, and intense salivation, followed by a period of depression and somnolence, which in turn gave place to another period of distress. The uneasiness gradually disappeared during a period of three to four hours, but depression was still evident five hours after the administration of the drug.

² Toxic dose of acetanilide, 0.2 gram per kilo. Vide, Sollman, Laboratory Manual of Pharmacology.

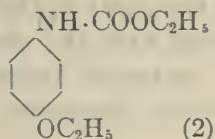
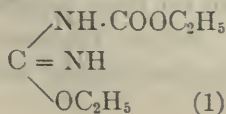
DISCUSSION OF RESULTS

The data obtained thus far, indicate that carbethoxy-ethyl-iso-urea possesses depressant properties of about the same order as those of urethane. Neither of these compounds approaches in power the cyclic ureides, barbital and luminal. This fact is doubtless related to the great stability of the latter compounds, both as regards their cyclic structure, and the carbon to carbon attachment of the alkyl groups. The relative ease of destruction of both urethane and the iso-urea compound would account for the speedy disappearance of depression after moderate doses.

The temperature effects observed are of especial interest in that they suggest an antipyretic action which is probably independent to some extent of the depressant action. It seems to be generally held that the fall of body temperature under the action of depressant drugs is largely due to diminished heat production, consequent upon loss of muscle tonus, and depressed general metabolism. It is, however, also recognized that increased heat loss is partly responsible for the fall of temperature since superficial vaso-dilatation is known to occur (2).

In the case of the aromatic antipyretics, it is fairly well established that the fall of temperature is mainly due to an increased heat loss, resulting from an action on the central heat-regulating mechanism, which manifests itself in appropriate vaso-dilator effects (3). The relatively large and rapid fall of temperature in normal rabbits obtained with carbethoxy-ethyl-iso-urea also suggests an increased heat loss as the dominant factor, whatever the nature of the action on the nervous mechanism may be.

In recent years a number of aromatic urethanes have been prepared and used successfully as antipyretics (4). Of these para-ethoxy-phenyl-urethane is well known, (the acetyl derivative is sold as "thermodin"), and it may be interesting and suggestive to compare the structure of this substance (2) with that of carbethoxy-ethyl-iso-urea (1):



A number of questions suggest themselves. Is there any relationship between the antipyretic action of these two compounds, and the possession of ether and urethane groupings? Is the benzene nucleus in (2) merely a stable carrier for active side chains? Is the mechanism of antipyresis the same for both compounds, and what is the nature of the mechanism in any case?

In an attempt to answer these questions, at least in part, the effect of the carbethoxy-iso-urea on oxygen consumption is being studied by the writer, and a number of urethanes of different types are being prepared for comparative studies along the same line. The result of these studies will be reported later.

Finally, with regard to the effects of carbethoxy-iso-urea on muscle, no definite explanation can be given as yet, though one or two suggestions may be offered. It is possible that, in response to the rapid fall of temperature, the muscle rigor develops as a protective mechanism to increase heat production. Shivering and rigor have been observed when a rapid fall of temperature has been induced by the coal-tar antipyretics (3).

A second explanation is suggested by the chemical similarity of the ethyl-iso-urea to guanidine. The latter has been shown to produce its tetanic effects through action on the myo-neural receptor (5), and it would seem worthwhile to investigate the possibilities of a similar mode of action in the case of the iso-urea compounds. The suggestion of such a comparison is also supported by a recent study of Klinger (6) who found that guanidine, and methyl-guanidine, administered to cats, produced nausea, salivation, and motor and psychic irritability, a condition somewhat resembling that of the cat under the influence of carbethoxy-ethyl-iso-urea. Comparative studies with guanidino-esters are being undertaken in the writer's laboratory as a further contribution to this problem.

I wish to express my thanks to Professor Julius Stieglitz for suggesting the study of the iso-ureas, and for his constant advice in connection with the chemical work; and to Professor A. L. Tatum for his expert direction of the pharmacological work, and much profitable discussion.

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THE COMPARATIVE STIMULANT EFFICIENCY OF OF SOME LOCAL AND SYSTEMIC AGENTS ON NOR- MAL AND DEPRESSED RESPIRATION, AND IRRI- TANT EFFICIENCY OF SOME AGENTS

P. J. HANZLIK

*From the Department of Pharmacology and Therapeutics, School of Medicine,
Leland Stanford Junior University, San Francisco*

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Aside from clinical impressions, practically nothing is known of the comparative value of the different agents in current use for reflex stimulation of the respiratory center, as in shock, syncope, accidents of anesthesia, resuscitation, etc. As a result, divergent views regarding the value of these agents exist in text books of pharmacology and therapeutics and in practice. It was, therefore, thought desirable to test out a number of the more commonly used agents on animals with normal and depressed respiratory centers in order to ascertain their value as respiratory stimulants and how the results measure up with clinical impressions. For comparative purposes, a few experiments were made with the more commonly used systemic agents which act on the center directly.

The results obtained with the reflex stimulants serve a double purpose. That is, besides evaluating respiratory stimulant efficiency they also give an idea of the irritant efficiency of the different agents that were studied. In fact, it is believed that the method of study used is peculiarly suited for certain agents whose irritant qualities would be difficult to evaluate by other methods.

The results are presented briefly in three parts. Part I will be presented first and deals with the method and reflex effects of locally acting agents on the normal and depressed respiratory

center. Part II deals with the systemic agents and part III presents a summary of the comparative irritant efficiency of the various agents.

PART I

1. Method

The method used is not new and consists of subcutaneous injection of the irritant, using the changes in respiration as an index of irritant and stimulant activity. The chief disadvantage of the method is the variability in the respiration of rabbits. The errors from this factor are reduced to negligible proportions by making a sufficiently large number of trials on different animals. This was done, of course, and only definite and unmistakable changes were accepted. In this way, I have compared the respiratory effects of a number of commonly used agents with a remarkable degree of constancy.

The experiments were performed on 20 rabbits using 18 different agents. In 10 of the rabbits the respiration was previously depressed by toxic doses of morphin, with the idea of imitating more or less the depressed (abnormal) functional state of the respiratory center which may be encountered in shock, syncope and analogous conditions. The animals were tied to boards and the respiratory movements were registered on a slow moving kymograph by three procedures: First by a tambour attached to a face mask made from a rubber ball and placed over the nostrils and mouth; second, by a tambour attached to a pneumatic rubber-bag placed over the abdomen and chest and covered with a bridge of galvanized iron (Cushny's method); and third, by a thoracograph stile resting on the chest and recording directly on the kymograph. The locally acting agents were injected in the dosage of 1 cc., using different areas of the abdomen for injection so as to avoid fatigue or depression of the sensory endings. The dosage of the systemic agents will be indicated separately. The results on respiration may now be described.

2. *Locally acting agents*

Normal respiration. The results of 78 trials with 18 different agents are presented in table 1. These are tabulated as to medians of maximal percentage changes in respiratory rate and amplitude and duration of action in minutes, including the range of changes for each agent, and arranged in the order of descending efficiency. This gives so good an idea of the effects of the different agents that were used as to justify the omission of a detailed description of each agent. Nothing would be gained by a recapitulation of the results in descriptive form. Instead, a brief, concise summary will suffice. However, before proceeding to this it should be stated as is obvious from the tabulated results, that the effects of the different agents varied and that they occurred almost immediately after injection, and also disappeared rather promptly. As soon as the respiration returned to the previous condition (control), another agent was injected. The medians of the maximal percentage changes in rate and amplitude produced by, and the durations of action (in minutes) of, the different agents were used for the estimation of their respiratory stimulant and irritant efficiencies, assigning the value of 1 to camphor oil (20 per cent) as the standard. These are presented in the last column of the table and represent grand medians of the medians for each factor used as index of respiratory stimulation. In this way, the results are expressed more concisely, conservatively and accurately. Naturally, the most efficient agent produced the greatest median percentage increase in rate, amplitude and longest duration of stimulation, and the least efficient agent produced opposite effects. Between these two extremes lay the intermediate effects consisting of corresponding degrees of stimulation of rate and amplitude and duration, or the disproportionate distribution of the effects. For instance, if an agent merely increased the rate temporarily, leaving the amplitude unchanged, such an effect would be equivalent to lessened efficiency as compared with one that increased the rate for a long period with amplitude unchanged, or one that also increased the amplitude in addition to the above effects. The results in table 1 show that the effects from the

TABLE 1
Summary of respiratory changes from various agents in untreated rabbits

AGENT	NUM- BER OF RAB- BITS USED	MEDIAN OF MAXIMAL PER CENT CHANGE IN RATE*	MEDIAN OF MAXIMAL PER CENT CHANGE IN AMPLITUDE*	MEDIAN DURATION OF ACTION	GRAND MEDIAN RESPIRA- TORY STIMULANT EFFICIENCY WHEN CAMPHOR OIL (30 PER CENT) = 1
Locally acting agents					
Introduction of hypodermic needle alone.	3	0 (0 to +6)†	0	minutes Momentary to 1	0
Water at 2° to 6°C.....	1	+71	+100	$\frac{1}{2}$ ($\frac{1}{8}$ to 4)	3.0
Water at 80°C.....	4	-28 (-62 to -7)	+79 (0 to +150)†	$1\frac{1}{2}$ ($\frac{5}{8}$ to 2)	2.6
Water at 10° to 15°C.....	6	+43 (+15 to +75)	+68 (+30 to +300)	$\frac{1}{2}$ ($\frac{1}{16}$ to 7)	2.2
Water at 18° to 20°C.....	4	+20 (+8 to +40)	+50 (+46 to +100)	1	1.3
Silver nitrate 1 per cent.....	3	+22 (-10 to +82)	+50 (0 to 100)	9 (4 to 13)	1.3
Mercuric chloride 0.1 per cent.....	3	+20 (+3 to +30)	+50 (-20 to +50)	$3\frac{1}{2}$ (2 to 6)	1.3
Chloroform.....	4	+19 (0 to +57)	+35 (0 to +150)	2 ($\frac{1}{2}$ to 3)	1.2
Formaldehyde 1 per cent.....	2	+17 (-5 to +22)	+6 (0 to +12)	$9\frac{1}{2}$ (9 to 10)	1.1
Camphor oil (20 per cent).....	3	+15 (0 to +20)	+30 (0 to +40)	$7\frac{1}{2}$ (5 to 10)	1.0
Tr. iodine (U. S. P.).....	3	+13 (-20 to +26)	+50 (-50 to +70)	8 (4 to 9)	1.0
Tannin 1 per cent.....	3	+13 (+4 to +40)	+35 (+25 to +46)	2 (2 to 17)	0.9
Oil of turpentine.....	4	+22 (0 to +33)	+24 (-20 to +50)	$2\frac{1}{2}$ ($1\frac{1}{2}$ to 3)	0.8
Ether.....	4	+12 (+8 to +23)	+20 (0 to +200)	$\frac{3}{4}$ ($\frac{1}{2}$ to 6)	0.8
Water at 60°C.....	3	+7 (-5 to +11)	+25 (+8 to +25)	1 ($\frac{1}{2}$ to $1\frac{1}{2}$)	0.8
Hydrochloric acid 1 per cent.....	3	+12 (+6 to +31)	+6 (+6 to +20)	5 (1 to $7\frac{1}{2}$)	0.6
Phenol 1 per cent.....	2	+8 (8)	+93 (-7 to +100)	$4\frac{1}{2}$ (4 to 5)	0.6
Ammonium hydroxide 5 per cent.....	3	+10 (-5 to +23)	0 (-16 to +3)	4 ($2\frac{1}{2}$ to 11)	0.5
Collargol 10 per cent.....	4	+4 (-23 to +13)	0 (-20 to +50)	$3\frac{1}{2}$ (1 to 12)	0.3
Alcohol 50 per cent.....	3	+8 (-30 to +12)	-20 (-20)	2 ($1\frac{1}{2}$ to 7)	0.2
Water at 40° to 50°C.....	4	+2 (-40 to +14)	+13 (0 to +25)	$\frac{1}{3}$ ($\frac{1}{3}$ to 5)	0.13
Alcohol 95 per cent.....	3	+18 (+14 to +20)	0 (0 to +7)	1 ($\frac{1}{2}$ to 1)	0.1
Water at 90°C.....	2	-5 (-10 to +5)	-5 (-25 to +20)	$2\frac{1}{4}$ (2 to $2\frac{1}{2}$)	0
Water at 30° to 38°C.....	3	0 (-3 to +15)	0	1 (0 to 3)	0
Sodium hydroxide 1 per cent.....	3	-6 (-7 to -5)	-25 (-50 to -7)	7 (2 to 8)†	0

Systemic agents

Strychnin (0.2 cc. 0.1 per cent per kilogram).	3	+ 6 (+5 to +11)	+20 (+9 to +20)	15 (9 to 17)	0.7
Atropin (1 cc. 0.1 per cent per kilogram) ...	2	- 7 (-14 to 0)	-10 (-20 to 0)	6 (10 to 11)	0
Caffein (1 cc. 1 per cent per kilogram)	3	0 (-7 to +30)	0 (-20 to 0)	(3 to 15)†	0
Morphin (toxic dose, 0.5 cc. to 0.75 cc. 4 per cent per kilogram)	9	-50 (-16 to -90)	-33 (-75 to +40)	25 (4+ to 51)	0

* + = increase ; - = decrease.
† Range in parentheses.
‡ Observed during this period only.

prick of the hypodermic needle are negligible, and therefore, the changes produced were due to the agents themselves.

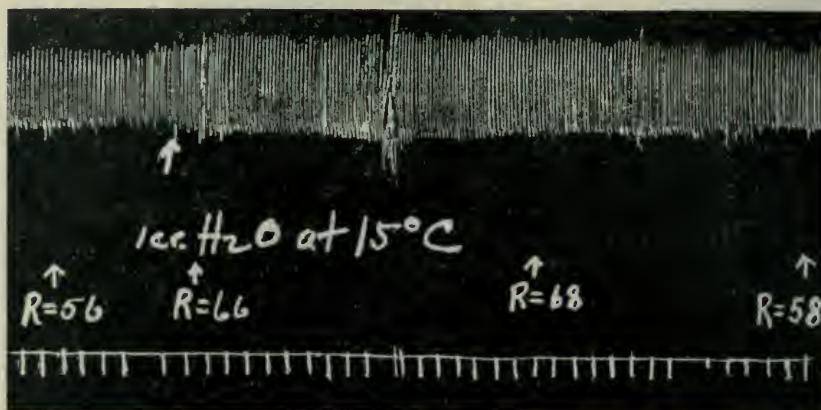


FIG. 1. EFFECTS OF WATER AT 15°C. (SUBCUTANEOUSLY) ON RESPIRATION OF NORMAL RABBIT (1.7 KG.)

R = Respiratory rate. Time, each stroke = five seconds

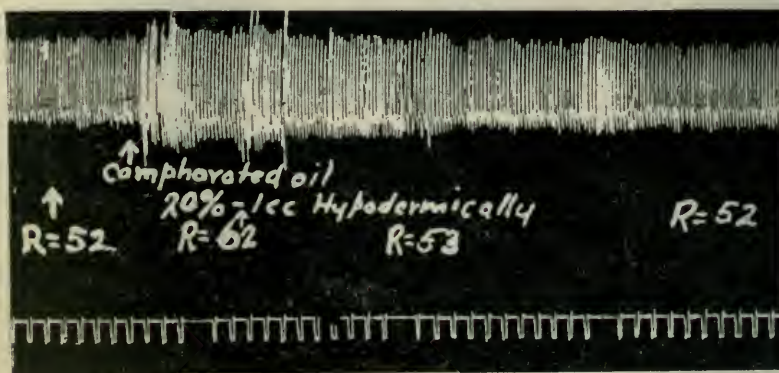


FIG. 2. EFFECTS OF 20 PER CENT CAMPHOR OIL (SUBCUTANEOUSLY) ON RESPIRATION OF NORMAL RABBIT (1.7 KG.)

R = respiratory rate. Time, each stroke = five seconds

The descending order of stimulant efficiency for normal respiration was found to be as follows: Cold water (2° to 20°); water at 80°C.; silver nitrate 1 per cent; mercuric chloride 1 per cent;

chloroform and formaldehyde 1 per cent; camphor oil (20 per cent) and Tr. iodin (U. S. P.), tannin 1 per cent, turpentine, ether and water at 60°, hydrochloric acid 1 per cent, phenol 1 per cent and ammonium chloride 5 per cent, collargol 10 per cent, alcohol 50 per cent, water at 40° to 50°C. and alcohol 95 per cent. Water at 90°C., water at 30° to 38°C. and sodium hydroxide 1 per cent were ineffective, and if anything, tended to decrease the rate and amplitude of respiration. The tracings in figures 1 and 2 illustrate typical effects obtained with the strongest stimulants, i.e., water at 15°C. and camphor oil (20 per cent).

Of all the agents tested, cold water was found to be the most efficient reflex stimulant for the respiratory center and, therefore, also most irritant locally. Warm and hot water were inefficient. Thus the relative merits of hot and cold water as reflex stimulants appear to be settled in favor of cold water. As would be expected, water from 30° to 38°, i.e., in the vicinity of body temperature, was inefficient. Water at 20°C. or less represented one extreme in stimulant efficiency which was chiefly reflected in the increases in rate and amplitude with some sacrifice of duration of stimulation. On the other hand, at the other extreme, i.e., water at 61° to 80°, the stimulant efficiency was mainly reflected in depth, the rate and duration of action being sacrificed. This is shown by the curves in figure 3. Cold 0.9 per cent sodium chloride solution gave similar results in one animal, so that the effects of water are concerned with temperature rather than hypotonicity. What relation these results possess to the distribution and specific response of the "cold" and "hot" nerves of the skin cannot be said. The reactions also do not conform strictly to the "protopathic" and "epicritic" senses of Head in human individuals. For instance, the protopathic sense in my experiments was not correctly evoked owing to the absence of reaction to extreme heat (water at 90°C). Likewise the lack of response to moderate degrees of heat (40° to 50°C) and cold (30° to 38°C) would eliminate the epicritic sense.

Although silver nitrate, mercuric chloride, chloroform and formaldehyde were found to be superior to camphor oil as reflex respiratory stimulants, they cannot be used in practice for

this purpose. On repeated administration, the metallic salts and formaldehyde would be too destructive to tissue and chloroform is too depressant for the higher functions. Tr. iodin (U. S. P.) was found to be as good a stimulant as camphor oil and tannin

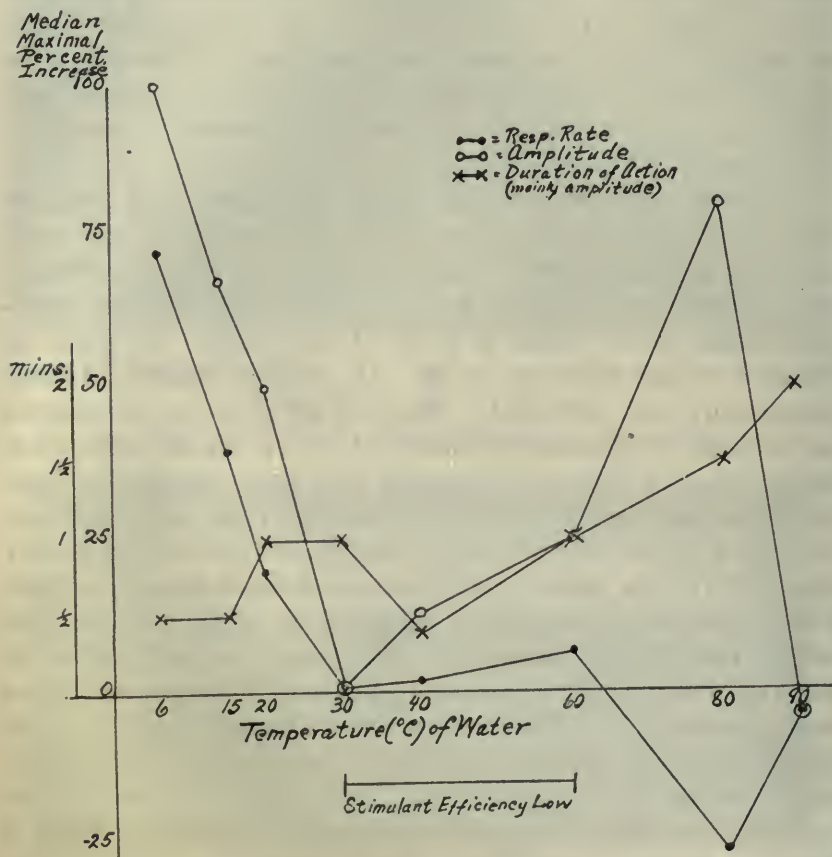


FIG. 3. RESPIRATORY STIMULANT EFFICIENCY OF WATER AT DIFFERENT TEMPERATURES INJECTED HYPODERMICALLY IN RABBITS

was a close second. However, neither iodine nor tannin has practical possibilities as a respiratory stimulant. The results with camphor oil sustain favorable clinical opinion and usage. Turpentine, ether and alcohol have been used subcutaneously for reflex stimulation, but they were found to be inferior to

camphor oil and certainly much inferior to water at 2° to 20°C. As far as ether and alcohol are concerned, the relatively poor results obtained by me confirm the unfavorable impressions of clinicians generally about these agents as respiratory stimulants. The remaining agents are too inefficient and objectionable as reflex stimulants and may be dismissed without further consideration. Of all the agents that were tested, water, or saline solution (0.9 per cent NaCl), at 2° to 20°C. and camphor oil (20 per cent) are most efficient and least objectionable. Cold water would be more suitable for sudden and powerful, though short, stimulation of the respiratory center, while camphor oil would produce moderately strong, but more sustained stimulation. Externally cold water is known to be quite efficient as a reflex stimulant, and there is no good reason why cold sterile water in small doses could not be used hypodermically. Economy and easy availability are obvious advantages over camphor oil, which has been reported to cause tumor formation.

Depressed respiration. The results of the experiments presented in table 2 were tabulated and respiratory stimulant efficiency estimated in the same way as those of table 1. They are arranged in descending order of efficiency and were obtained on 10 animals, injected with toxic doses of morphin (0.5 cc. and more of 4 per cent per kg.) before the experiments were begun. The respiration was definitely and markedly slowed by the morphin in all of these animals, and there was also considerable motor depression. The functional state of the respiratory center in morphinized animals was analogous to that in shock and comatose conditions. The results that were obtained with a number of agents commonly used as resuscitative measures (except water and nicotin), and which also were used in the untreated animals, are rather interesting. In fact, the position of certain agents as to respiratory stimulant efficiency is reversed. Beginning with the most efficient first, the order is as follows; camphor oil, chloroform, alcohol and water at 2° to 90°C., inclusive, and ether, which was ineffective. It is rather interesting that cold water should occupy a distinctly inferior position when the respiratory center is depressed. Chloroform was found

TABLE 2
Summary of respiratory changes from various agents in morphinized rabbits

AGENT	NUM- BER OF RAB- BITS USED	MEDIAN OF MAXIMAL PERCENT CHANGE IN RATE*	MEDIAN OF MAXIMAL PERCENT CHANGE IN RATE*	MEDIAN DURATION OF ACTION	GRAND MEDIAN RESPIRA- TORY STIMULANT EFFICIENCY WHEN CAMPHOR OIL (20 PER CENT) = 1
Locally acting agents					
Camphor oil—20 per cent.	5	+77 (+12 to +150)†	0 (-20 to +22)†	4 (1 to 20)†	1.0
Chloroform.	8	+30 (-50 to +190)	+7 (-12 to +33)	1 (1 to 4)	0.4
Alcohol 95 per cent.	1	+30	+40	1	0.4 (?)
Water at 2°C.	6	0 (-15 to +7)	+18 (+14 to +40)	$\frac{1}{2}$ ($\frac{1}{8}$ to 1)	0.1
Water at 10°C.	7	+7 (0 to +100)	+14 (0 to +50)	$\frac{1}{2}$ ($\frac{1}{8}$ to 2)	0.1
Water at 25°C.	11	+7 (-17 to +100)	+6 (0 to 50)	$\frac{1}{2}$ ($\frac{1}{8}$ to 2)	0.1
Water at 40°C.	7	0 (-31 to +54)	+10 (0 to +100)	$\frac{1}{2}$ ($\frac{1}{8}$ to 2½)	0.1
Water at 60°C.	8	+12 (0 to +41)	0 (-14 to +16)	$\frac{1}{2}$ ($\frac{1}{8}$ to 3)	0.1
Water at 80°C.	9	+16 (-23 to +43)	+8 (0 to 33)	$\frac{1}{2}$ ($\frac{1}{8}$ to 2½)	0.1
Water at 90°C.	6	+12 (-9 to +50)	0 (-16 to +33)	$\frac{1}{2}$ ($\frac{1}{8}$ to 3)	0.1
Ether.	7	0 (-60 to +63)	0 (-14 to +40)	1 (1 to 10)	0
Systemic agents					
Strychnin (0.2 cc. of 0.1 per cent per kilogram)	3	+25 (-25 to +25)	+20 (-20 to +20)	4 (4 to 8)	1.0
Atropin (1 cc. of 0.1 per cent per kilogram).	5	+20 (0 to +100)	0 (-33 to +200)	38 (6 to 50)	1.0
Nicotin (0.5 cc. of 0.1 per cent per kilo- gram)	6	+15 (-40 to +120)	-25 (-50 to +50)	25 (5 to 33)	0.2

* + = increase; - = decrease.

† Range in parentheses.

to be less than half as efficient as camphor oil for a depressed than normal respiratory center, while ether was ineffective, being moderately effective for the normal center. In the one trial that was made, strong alcohol was no more efficient than ether. The results with chloroform and ether confirm the unfavorable clinical impressions generally held about these agents as reflex respiratory stimulants in collapse conditions. Out of all the agents that were tried, camphor oil (20 per cent) is the only one that retained its position as a good reflex stimulant for the respiratory center, whether normal or depressed. Figure 4 illustrates the action of camphor oil in a morphinized rabbit.

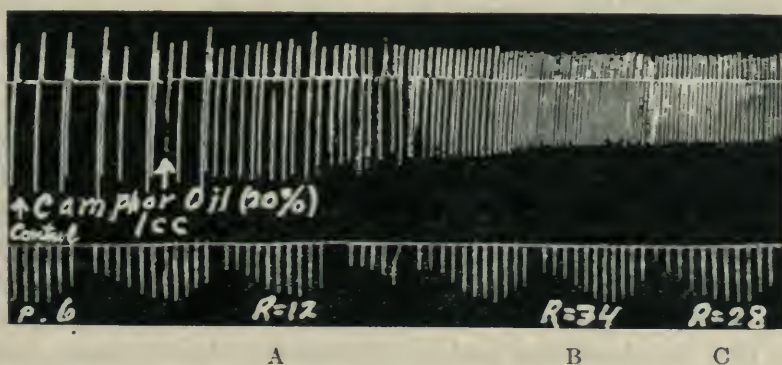


FIG. 4. EFFECTS OF 20 PER CENT CAMPHOR OIL (SUBCUTANEOUSLY) ON MORPHINIZED RABBIT (1.5 KG.)

R = respiratory rate. Toxic dose of morphin was used; i.e., 0.75 cc. of 4 per cent per kilo subcutaneously; at end of twenty minutes respiration was slowed from 48 to 6 per minute. A, Control and immediate stimulant action of camphor oil. B, End of nine minutes, and C, end of eighteen minutes respiration continues to be stimulated. Time, each stroke = five seconds.

Whatever the explanation of these results may be, they serve to illustrate, at least, that the action of drugs in altered functional states, (here the respiration) is not necessarily the same as on the normal mechanism. The actions of the majority of the agents were not exerted on the respiratory center directly, but on functions which excite the center indirectly or reflexly, that is, the sensory mechanism beneath the epidermis. To what extent this mechanism was influenced by the morphin is not known, although it may be regarded as practically negligible.

It would appear that the depressed center would require more powerful stimuli than the normal center to evoke a given response. Accordingly, cold water, which was found to be such a strong stimulant for the normal center, would be expected to occupy a closer position to other good stimulants. However, this was not found to be the case. Hence, the conclusion is forced that this unusual response was brought about by an altered functional activity of the respiratory center, supplemented, in part perhaps, by altered activity in the sensory mechanism. However, until the physiology of the sensory mechanism, including the epicritic and protopathic senses, is better understood it is useless to speculate further.

PART II

Systemic agents

In order to make the study more complete, and for purposes of comparison, the following systemic agents were tested out in the same way as the locally acting agents just described; strychnin, caffein, atropin and nicotin. Of these, strychnin, caffein and atropin are used therapeutically. Greater intervals of time between injections were allowed with these agents so as to give time for absorption and development of their action. As a rule, this amounted to not less than fifteen minutes. First, the results on the normal respiration will be summarized.

Normal respiration. The results of 20 different trials with strychnin, atropin and caffein are presented in table 1. The order of respiratory stimulant efficiency was determined in the same way as with the locally acting agents, assigning the value of 1 to camphor oil (20 per cent). According to this method of study, strychnin was found to stimulate respiratory activity invariably, although to different degrees in the three animals that were observed. Its stimulant efficiency was found to be about $\frac{1}{10}$ of that of camphor oil, therefore, quite good. On the other hand, caffein was ineffective, and atropin somewhat impaired, if anything, the respiration and, therefore, also the efficiency. The poor results obtained with atropin and caffein, and, in part, with strychnin, as respiratory stimulants confirm

the general tendency of the results of Loevenhart, Malone and Martin (1) with these agents when compared with sodium cyanide intravenously in dogs. Sodium cyanide was not used in my experiments because it is unsuited for respiratory stimulation by hypodermic administration, which was used uniformly throughout my work.

As far as the normal respiratory center is concerned, the results obtained show conclusively that the locally acting, or reflex stimulants, that were used are superior to the systemic stimulants. This agrees generally with current physiological conceptions of the comparative efficiency and value of reflex and direct stimulations for the respiratory center. However, the results of the experiments here reported do not altogether sustain this notion when the center is previously depressed by morphin.

Depressed respiration. Fourteen trials were made with strychnin, atropin and nicotin on different rabbits previously injected with toxic doses of morphin in the same way as in the experiments with locally acting agents. The drugs were injected subcutaneously in doses generally corresponding to, but sometimes even greater than, the therapeutic dosage for man. The results are presented in table 2 in the same way as all other results of this paper.

Using camphor oil (20 per cent) in morphinized animals as the standard, it is seen that both strychnin and atropin are equally efficient and as good as camphor as respiratory stimulants. The results with these stimulants were different in normal (unmorphinized) animals. That is, strychnin was only $\frac{7}{10}$ as efficient as camphor in normal animals and atropin was ineffective. My results with atropin confirm the statement of Meyer and Gottlieb (2) that this drug is particularly efficient as a respiratory stimulant in morphin poisoning. Caffein was not tried in morphinized animals. Nicotin was found to possess only $\frac{1}{3}$ the efficiency of camphor oil (20 per cent). These results, therefore, confirm the same general tendency exhibited by the locally acting or reflex stimulants on the normal as compared with the depressed respiratory center. That is, a change in the functional state of the center altered the response of the center

though not as completely with the systemic as with the locally acting agents. For the systemic agents, the depressed center seemed to be made somewhat more sensitive to the action of strychnin and distinctly more sensitive to atropin. In a sense, there was a reversal in the action of atropin, because this alkaloid actually depressed the respiration in normal animals, while it stimulated this function and increased its efficiency in morphinized animals.

PART III

Comparative irritant efficiency of various agents

The comparison of many irritant agents by precipitation of albumin, skin, blood, etc., in vitro is impossible, because they do not precipitate protein effectively or at all. This applies to cold water, ether, chloroform, camphor oil and some other agents, which are used for their irritant properties with varying success in therapeutics as reflex stimulants. Direct application to skin and judging the effects by the degree of inflammatory changes (rubefaction, swelling, vesication, pain, etc.), has its disadvantages even with those agents that can precipitate or penetrate the skin. Great variability in the functional and morphological characteristics of skin in different species and in different regions of the same individual is at once encountered. Human skin, although most desirable as a test object, is generally unavailable for extensive experiments. The skin of animals is certainly less satisfactory than that of man even when specially prepared by shaving. Blisters on animals are unobtainable, except possibly on horses, and the edema produced may be out of proportion to the degree of irritation.

The procedure used for estimating reflex respiratory stimulant efficiency in part I is suited for estimating irritant efficiency and possesses several advantages over the precipitation and contact skin methods. All agents, whether they precipitate protein or not, can be tested in this way. A great many agents can be tested on the same animal repeatedly. The unknown factors of variable thickness and uncertain penetration of skin are avoided. Vesicant qualities, of course, cannot be compared in

this way, but lack of knowledge concerning vesicant properties would not necessarily alter the classification or irritants obtained by this method. The effects are transmitted through the afferent or sensory paths whose end organs are located beneath the epidermis. It would seem, therefore, that, when irritants are brought in direct contact with the end organs by subcutaneous injection rather than upon the skin, this would give more constant and reliable results.

The results on normal respiration presented in Part I give an idea of the irritant efficiency of the locally acting agents that were used. In descending order the arrangement of the agents as irritants is exactly the same as their arrangement as respiratory stimulants and need not be repeated. Water from 2° to 20°C. comes first in order, and silver nitrate, mercuric chloride, chloroform and formaldehyde maintain their superiority over camphor oil (20 per cent). As far as metallic salts, formaldehyde and chloroform are concerned, their high irritant efficiency by this method is confirmative of the results by other methods. However, when it comes to water and camphor oil, the irritant efficiency of these agents could not at all be ascertained by protein precipitation in vitro, and doubtfully by skin irritation in vivo, all of which indicates the peculiar suitability of the method used in this paper for agents of this sort. As for the remaining agents, Tr. iodin and tannin are also efficient irritants, being about as irritating as camphor oil and this agrees in general with their irritant efficiency by other methods. The rest of the agents are all weaker, and this also agrees with what is known of their irritant qualities in other directions, except 95 per cent alcohol, which is a fairly good protein precipitant in vitro and for mucosal and dermal surfaces, but by the method of study here described, alcohol occupies a very subordinate position. Its precipitant properties in the subcutaneous tissue might be quite different from that of serum in vitro or on skin. Higher concentrations than were used of such agents as phenol, hydrochloric acid and sodium hydroxide would probably also modify their positions as irritants by this method. This would not be true of ether, turpentine, water at 30° to 60°C. and collargol. The

low position occupied by collargol as an irritant is confirmative of its inferiority as a protein precipitant *in vitro* observed by Sollmann (3).

From what has been said of the irritant efficiency of these agents, it is seen that the results obtained by the index of respiratory activity agree with those by other methods of study, especially protein precipitation *in vitro*, and that for some agents, notably water and camphor oil, the respiratory method is admirably suitable, establishing their superiority over other agents as irritants for living tissues.

CONCLUSIONS

1. Using changes in rate and amplitude, and duration of action as the index of respiratory activity, the following agents arrange themselves in descending order of stimulant efficiency for the respiration of normal or untreated rabbits; water at 2° to 20°C. (inclusive) and at 80°C., silver nitrate 1 per cent, mercuric chloride 0.1 per cent, chloroform, formaldehyde 1 per cent, camphor oil (20 per cent) and Tr. iodine (U. S. P.), tannin 1 per cent, turpentine, ether, water at 60°C., hydrochloric acid 1 per cent, phenol 1 per cent, ammonium hydroxide 5 per cent, collargol 10 per cent, alcohol 50 per cent, water at 40° to 50°C., alcohol 95 per cent, and water at 90°C., water at 30° to 38°C. and sodium hydroxide 1 per cent.

2. The respiratory results obtained with camphor oil, ether, chloroform and alcohol agree in general with clinical impressions about these agents.

3. The order of respiratory stimulant efficiency of the locally acting agents was different when the respiratory center was previously depressed by toxic doses of morphin, namely, in descending order, as follows; camphor oil (20 per cent), chloroform, water at 2° to 90°C., inclusive, and ether, which was inefficient.

4. Among systemic agents that were tried, strychnin, caffeine and atropin, in doses equal to and larger than therapeutic, were found to be distinctly inferior as respiratory stimulants as compared with camphor oil and water at 2° to 20°C., which were the most efficient of all agents studied.

5. Strychnin and atropin were found to be more efficient as systemic stimulants in morphinized than in normal rabbits.

6. These results support the notion that the altered functional activity of a mechanism (here the respiratory center) can alter its response to drugs and other agents, acting reflexly and directly.

7. Respiratory activity is a valuable index of local irritant efficiency. Therefore, the various agents in (1) occupy corresponding positions as local irritants.

8. The results as to irritant efficiency agree, in general, with those obtained by other methods of study in vitro and in vivo.

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STUDIES ON THE PHARMACOLOGY OF SODIUM CITRATE

I. THE INFLUENCE OF SODIUM CITRATE ON RESPIRATION AND CIRCULATION

WILLIAM SALANT AND NATHANIEL KLEITMAN

From the Department of Physiology and Pharmacology of the University of Georgia, Augusta, Georgia

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Certain aspects of the action of sodium citrate have been presented within recent years by one of us in collaboration with several of his associates. Its effect on the isolated heart of different animals was reported by Salant and Hecht (1); observations on its action on the isolated intestine were made by Salant and Schwartz (2); experiments on the toxicity and fate of sodium citrate were reported by Salant and Wise (3); and the influence of diet together with observations on tolerance and cumulation were studied by Salant and Swanson (4). The present series of reports is, therefore, a continuation of the previous investigations and includes studies with sodium citrate on respiration, circulation, intestinal movements, the central nervous system and the reversal of action after atropine and pilocarpine. Since the literature was extensively reviewed in the above reports, repetition in the present communication may be dispensed with. But those writings which are essential for the discussion of the results will be cited in the different parts of the present series.

THE INFLUENCE OF SODIUM CITRATE ON RESPIRATION

The literature on the pharmacology of sodium citrate contains but few observations on its action on respiration. Pommer (5) reported that it caused acceleration of respiration in animals.

Later Robertson and Burnett (6) stated that when citrate was injected subcutaneously into rabbits, it produced dyspnea. In a more recent investigation by Salant and Wise (3) it was found that the subcutaneous injection of 1 gram of sodium citrate into rabbits accelerated respiration, but the same or even smaller amounts given intravenously caused dyspnea, Cheyne-Stokes respiration, and finally paralysis of the respiratory center. In experiments on other animals similar effects were produced. Large doses given subcutaneously to cats caused dyspnea. Intravenous injection of sodium citrate into dogs caused arrest of respiration with recovery, when medium doses were given, and paralysis when these were repeated or when a single dose was sufficiently large. Owing to the obviously fragmentary character of the information on the subject a reinvestigation of the action of sodium citrate was undertaken. The results obtained are presented in the following pages. The experiments were carried out on cats, dogs and rabbits. Urethane anesthesia was employed in experiments on cats and rabbits, but for dogs chloretone or morphine-ether were also used. To eliminate the influence of anesthesia observations were made on the effect of citrate on unanesthetized as well as on decerebrated animals.

Experiments on dogs

It may be stated at the outset that small and medium doses of sodium citrate injected intravenously usually stimulated respiration in all animals. The response was prompt and vigorous. Respiration was increased in frequency and also became much deeper. A striking illustration of the action of citrate on respiration in dogs was furnished by an experiment on a young animal (urethane anesthesia) which received 30 mgm. of the salt per kilo intravenously, shown in figure 1. Though the effect of repetition of the same dose was less pronounced, it was nevertheless considerable. That the anesthesia employed was an important factor in determining the action of citrate was shown when we changed from urethane to chloretone and to morphine-ether. The action under chloretone anesthesia was very inconsistent. Doses of 22 to 45 mgm. citrate per kilo injected intra-

venously sometimes caused stimulation and sometimes depression of the respiration in different animals, and in one experiment the breathing became deeper, but was less frequent. The effect also varied, when citrate was administered to dogs under morphine-ether anesthesia. Small doses of about 30 mgm. per kilo did not produce any change at first, while later injections of the

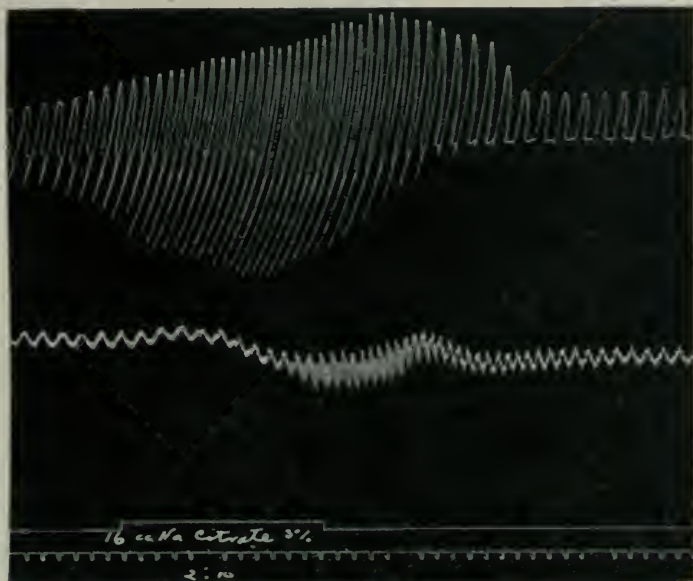


FIG. 1. EFFECT OF CITRATE ON RESPIRATION AND BLOOD PRESSURE IN DOG

Experiment 164. Dog, Skilos. Urethane anesthesia. Upper tracing, respiration, obtained by recording changes in intrathoracic pressure. Lower tracing, blood pressure, recorded by mercury manometer. Injection of 60 mgm. sodium citrate per kilo stimulated respiration, lowered blood pressure and slowed the heart. Time five seconds. Reduced one-half.

same amounts caused depression. We also observed in several cases that respiration may be stimulated in some individuals by doses which were ineffective in others. In all experiments, however, depression and paralysis occurred when the dose was increased to about 1 decigram per kilo. If the dose, whatever its size, was given repeatedly, it was either without effect on respira-

tion, or it depressed respiration even when urethane was used as an anesthetic. With doses of larger size the rate of respiration was reduced from 30 to 10 per minute.

Experiments on cats

Respiration was also stimulated in cats after the administration of citrate, when sufficient amounts were injected. Small doses, 7 to 15 mgm. per kilo injected intravenously were without any effect on respiration. But when the amounts were increased to 22 to 100 mgm. citrate per kilo, and injected with moderate speed, respiration was stimulated, usually becoming greatly increased in depth and frequency. But the stimulation thus produced seldom lasted more than two or three minutes. Occasionally no change in frequency occurred, but respiration was greatly increased in depth. Although the effect of a single dose was quite constant, that of repeated doses was not uniform. The response also varied in different individuals which was probably due to variation in the rate of oxidation and elimination observed by Salant and Wise (3).

The effect on respiration in some cats showed a steady diminution with each succeeding dose, though this was given at intervals of about fifteen minutes and was only 30 mgm. per kilo. But in other experiments doses of 60 to 70 mgm. per kilo administered at intervals of ten to thirty minutes and injected with considerable speed caused, on the contrary, greater stimulation of respiration when the injection was repeated. Still larger doses also stimulated respiration, but this was transitory, and was followed by prolonged depression which increased upon the repetition of the dose. The double action of citrate was obtained with continuous and slow injections when given at the rate of 1 cc., 30 mgm. per minute. Stimulation occurred at first, but this gradually changed to depression which persisted when 300 mgm. per kilo were injected.

Intramuscular injections of citrate were made in two experiments. Two grams each were given to a dog weighing 8 kilos and to a cat weighing 2.75 kilos. Respiration was stimulated shortly after the injection in both animals. This subsided, how-

ever, in the dog, while in the cat respiration remained more rapid and deeper, though not to the same extent as immediately after the injection.

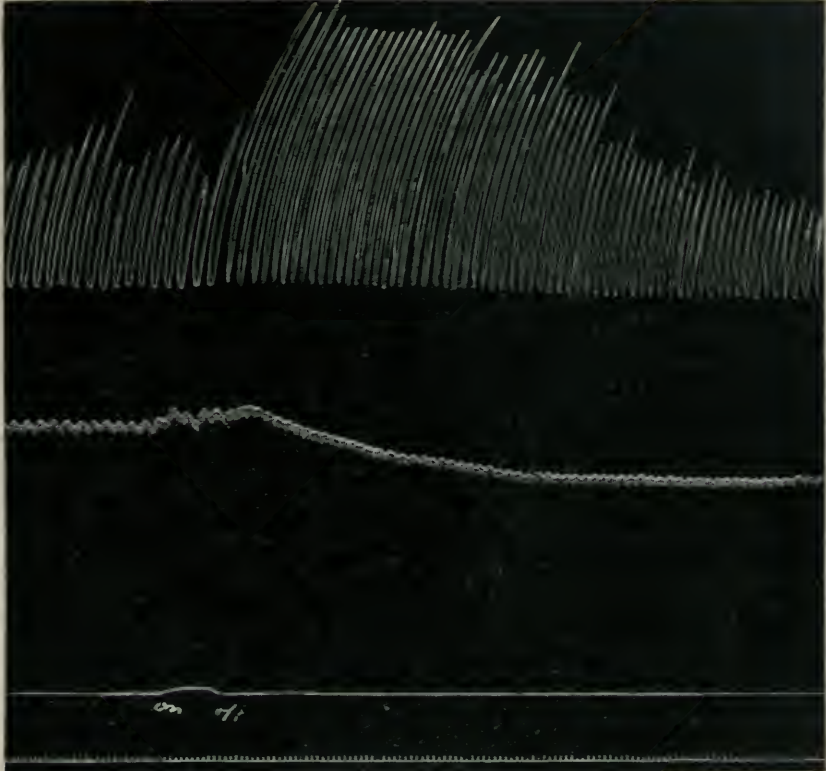


FIG. 2. EFFECT OF CITRATE ON RESPIRATION AND BLOOD PRESSURE IN CAT

Experiment 68. Cat, 3.2 kilos. Urethane anesthesia. Upper tracing, respiration, recorded by modified pneumograph method. Lower tracing, blood pressure, recorded by mercury manometer. Injection of 30 mgm. sodium citrate per kilo stimulated respiration, and lowered blood pressure. Time one second. Reduced one-half.

Observations were also made on decerebrated cats. The cerebrum was removed under ether anesthesia and three to four hours later 2 grams per kilo of sodium citrate were injected subcutaneously in three cats and intramuscularly into one.

Acceleration of respiration occurred in all our experiments. The rate of respiration was increased in one cat from 10 to 20, and later to 32, per minute, in another from 20 to 50, but in the third in which the rate of respiration before citrate was 32 the acceleration was only 25 to 30 per cent. The effect became noticeable within twenty to thirty minutes after the injection. When given into the muscles, the maximum increase in rate, which was 50 per cent, was observed within nine minutes after the injection. Respiration also became deeper in these animals. This observation was also previously made by us on a decerebrated cat which received citrate intravenously.

Experiments on rabbits

Three rabbits under urethane anesthesia were given a number of injections of sodium citrate into the jugular vein. The first three injections of 30 mgm. per kilo each given respectively at intervals of twenty-two and thirty-eight minutes increased the rate of respiration in one rabbit about 100 per cent. But when the same dose was repeated eight minutes later and given with approximately the same speed as the preceding, it produced transitory acceleration followed by slowing of the respiration lasting about one minute. A fifth test made after a lapse of thirty-seven minutes caused arrest of respiration in the expiratory phase for about fifteen seconds, and this was followed by a considerable decrease in frequency and a pronounced increase in amplitude. When thirteen minutes later a dose of 60 mgm. citrate per kilo was injected, respiration became irregular at first, then stopped. The total amount injected was 210 mgm. per kilo and was given in one hour and fifty-seven minutes. The response to citrate was at first the same in another rabbit, but subsequent injections have shown that its action may vary with the frequency of administration. Hence, when sufficient time between injections was allowed, respiration was accelerated, though the same amounts given previously caused depression. We may add in this connection that a noticeable difference was observed between cats and dogs on the one hand and rabbits on the other. The latter recover more readily from the depressing

effects of citrate than the carnivora, which may be explained by the difference in the rate of oxidation of the salts, as shown by Salant and Wise (3).

Effect of vagotomy

Experiments were also carried out on the action of citrate on respiration after the division of both vagi in dogs and cats. Only a slight increase in rate and amplitude occurred in one experiment. The effect of the same dose, 30 mgm. citrate per kilo, in another was not constant, respiration being sometimes accelerated and sometimes retarded, but the amplitude was always increased. Stimulation was marked in a third experiment, but this was less than before double vagotomy, while in a fourth citrate caused depression of respiration, when injected after division of the vagi. The effect of vagotomy on citrate action was also studied in a decerebrated cat. Citrate injected intravenously with both vagi intact increased the frequency and depth of the respiration. After division of both vagi the same dose of citrate increased the amplitude, but respiration was very much slower. Slowing also occurred in two other experiments in which citrate was injected after atropine and double vagotomy. The amplitude was much increased in one, but was diminished in the other. It is evident from the data presented above that the effect of citrate on respiration is modified by eliminating the vagi. A tendency to slowing or to slight stimulation only may be regarded as the main effect produced when citrate is given to animals after double vagotomy.

THE INFLUENCE OF SODIUM CITRATE ON THE CIRCULATION

The object of these experiments was to determine the action of sodium citrate on blood pressure, to ascertain its effect on the heart in intact animals, and to gain further information concerning its action on the isolated heart. The experiments were performed on different animals and under the same conditions as described in the preceding section. In all the experiments in which intravenous injections were made a 3 per cent solution of sodium citrate was used. (The pure salt which we used was

kindly obtained for us by Mr. W. C. Emerson of the Department of Chemistry by recrystallization from the C. P. product purchased in the open market.)

Blood pressure: Experiments on dogs

The changes in blood pressure produced by citrate in dogs varied with the size of the dose, the speed of injection and the anesthetic employed. Small doses, 20 to 30 mgm. per kilo, given rapidly by vein, failed to cause marked disturbance of the circulation in dogs under chloretone anesthesia. A fall of about 10 mm. Hg with prompt recovery occurred in some experiments, while in others blood pressure was not at all affected by these amounts of citrate. Circulatory depression occurred only when the doses were considerably increased in size (about 100 mgm. per kilo), this effect being produced even when the salt was introduced slowly into the circulation. As the resistance might be due to the low blood pressure which prevailed under chloretone anesthesia, the action of citrate was tested in another series of experiments in which urethane was the anesthetic used. Although the blood pressure before citrate was considerably higher, its action was practically the same as under chloretone anesthesia. Either no effect at all could be noticed with the smaller doses though the injection was rapid, or blood pressure fell about 20 mm. Hg (15 to 20 per cent) from which it recovered in about one minute. When larger amounts in proportion to body weight were given, similar effects were noticed. The difference in the fall of blood pressure was not pronounced, though the dose was doubled. Nor did any considerable disturbance of the circulation occur, even when the injections were repeated at short intervals. We noticed in several experiments that after transitory depression blood pressure was, on the contrary, slightly elevated by the injection of citrate. The response to citrate in dogs under morphine-ether anesthesia was not constant. Doses of 50 to 80 mgm. of the salt per kilo caused a rise in blood pressure in some animals lasting several minutes, while the same amounts in others produced the opposite effect. That the differences in reaction

may be accounted for by changes in the irritability of the vagus mechanism was indicated by the following experiment.

Experiment 193. A dog weighing 5.5 kilos was given 10 mgm. morphine sulphate per kilo subcutaneously, and later ether. Several doses of 30 mgm. citrate per kilo produced marked slowing of the pulse, blood pressure being at the same time moderately depressed. The vagus pulse disappeared, however, when this dose was repeated a number of times. Heart action became much faster, the increase in rate being particularly marked with larger doses.

It may be added that when a single dose of about 100 mgm. per kilo or if a sufficient amount of citrate was introduced in divided doses, a fall in blood pressure and paralysis of the circulation occurred with all anesthetics.

Experiments on cats

A very extensive series of observations with citrate was made on cats to which different amounts were administered intravenously with varying speeds and at different intervals. As will be seen from the following description, the results obtained in these animals differed in several respects from the effects produced by citrate in dogs.

Depression of the circulation occurred in cats which received different amounts of citrate. Doses of 30 to 40 mgm. per kilo caused a fall of blood pressure of 15 to 36 per cent, and doses of 60 to 70 mgm. per kilo a fall of 28 to 66 per cent. The absolute decrease varied between 20 and 30 mm. Hg for small doses and between 35 and 75 mm. Hg for large doses. The previous condition of the blood pressure had no influence on the change in circulation produced by citrate, as the degree of depression was often the same, whatever the height of the blood pressure before the injection. The duration of the effect was usually several minutes. The low level of blood pressure reached after the injection lasted about one to three or four minutes, while the recovery following occupied three to nine minutes. After larger doses recovery occurred within about fifteen minutes. We would

also record that in exceptional cases little or no change was produced either by the small or large amounts of citrate, and that the low blood pressure caused by the salt may sometimes persist even when a small dose is given. Repetition of dose usually increased the depression and delayed the recovery, this being especially marked after larger doses, though the intervals between the injections were in some experiments about thirty minutes. The recovery from the effects of small and larger amounts of citrate when given rapidly and repeated at different intervals suggested testing its action when introduced slowly into the circulation. In one experiment on a cat weighing 1300 grams 13 cc. of 3 per cent citrate solution were injected in twenty-four minutes without producing any change in blood pressure. This experiment was repeated on another cat weighing two kilos, 20 cc. being injected in twenty-nine minutes. Blood pressure fluctuated during the injection and was considerably depressed after the first cubic centimeter of fluid was introduced. When the entire amount was given, blood pressure was about 30 per cent lower than at the beginning of the experiment. Gradual recovery occurred. In a third experiment the same amount per kilo of body weight was injected still more slowly. Nevertheless blood pressure fell more than in the previous experiment (from 140 to 70 mm. Hg), but gradual recovery also occurred in this case, and the blood pressure gained about 35 mm. Hg in twelve minutes after the injection. Two more cats received each 300 mgm. citrate per kilo in thirty and twenty-five minutes, respectively. Blood pressure fell 40 mm. Hg in one and 20 mm. in the other. Blood pressure increased about 10 mm. Hg in the first within twenty-five minutes, but practically no improvement occurred in the second during a period of observation of fifteen minutes after the injection.

Experiments on rabbits

The effect of citrate on blood pressure was also tested in three full grown rabbits under urethane anesthesia. Depression of the circulation was observed in each, when a dose of 30 mgm. citrate

per kilo was injected into the jugular vein. The fall in blood pressure was, however, very moderate (not exceeding 10 mm. Hg) and often transitory after the first few injections. This was preceded by considerable fluctuations of blood pressure and a vagus pulse immediately after a rapid injection. The depression persisted when five to six doses were injected, and especially when the above amount was doubled.

Intramuscular injections

Depression of the circulation also occurred in two experiments in which sodium citrate was injected into the muscles. Blood pressure slowly declined after a preliminary brief rise of a few mm. Hg. In one experiment on a dog which received 250 mgm. citrate per kilo into the gluteal muscles blood pressure was elevated 10 mm. Hg promptly after the injection. The fall that followed it amounted to 35 mm. Hg in one hour, which was about 16 per cent below the level before the injection. It may be noticed in this connection that notwithstanding the very moderate fall of blood pressure which took place after such a large dose, the injurious action of citrate on the heart manifested itself when one hour after the injection stimulation of the vagus promptly caused cardiac paralysis. Similar results were obtained in the other experiment on a cat, but though the dose was about three times as large as in the dog, blood pressure fell 30 mm. Hg during the first hour; a further reduction of 20 mm. Hg occurred during the next two hours, and paralysis of the circulation 3 hours and 11 minutes after the injection.

Mammalian heart

Evidence that the heart in situ was affected by citrate was first obtained by us in a series of experiments in which observations were made on changes in the volume of the kidney by means of an oncometer. With smaller, but especially with larger, doses the volume of the kidney usually followed the blood pressure, the diminution in volume which occurred during the depression of the circulation indicating that heart action was

affected by the injection of citrate. Its effect on the heart was further studied by means of a Cushny myocardiograph which was attached to the ventricles. A dose of 100 mgm. citrate per kilo given intravenously to a cat under urethane anesthesia produced arrest of the heart in diastole for about one and one-half minutes. Although considerable improvement occurred, heart

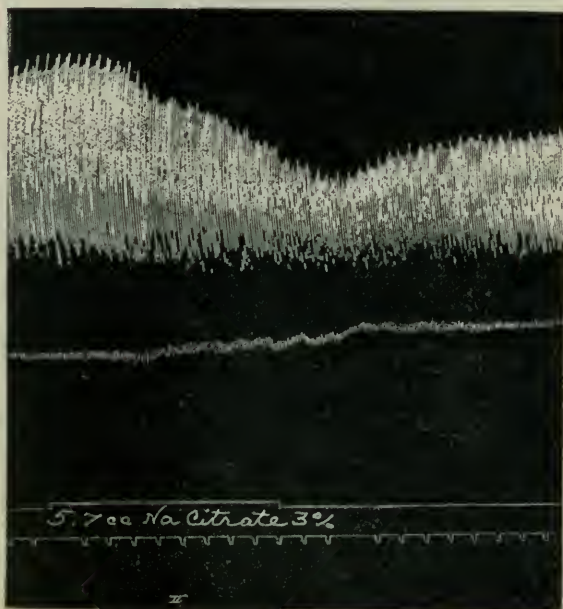


FIG. 3. EFFECT OF CITRATE ON HEART AND BLOOD PRESSURE IN DOG

Experiment 183. Dog, 5.7 kilos. Chloretone anesthesia. Upper tracing, heart, recorded by a Cushny myocardiograph attached to ventricles. Lower tracing, blood pressure, by mercury manometer. Injection of 30 mgm. sodium citrate per kilo depressed the heart. Blood pressure rose during and after the injection. Time five seconds. Reduced one-half.

action remained weak for some time after the citrate was injected. The depression produced was also marked when the amounts administered were much smaller. The amplitude was decreased in some experiments 45 to 50 per cent, or even more, when these amounts were repeated. Though the rate was also decreased, this was never pronounced. In fact, it was often

difficult to decide whether the frequency of the heart was changed by the injection of citrate. Substantially the same results were obtained in dogs, due allowance being made for individual variation. We would also state that a small dose of about 15 mgm. citrate per kilo failed to produce a noticeable effect on the heart.

In this connection mention should be made of the very interesting observation that though the heart was markedly depressed by citrate, blood pressure sometimes remained entirely unaffected, or even rose after the injection, indicating a very prompt compensatory vasomotor adjustment (fig. 3).

Isolated heart

Salant and Hecht (1) have shown that cardiac depression usually resulted, when the isolated heart of frogs, cats and dogs was perfused with sodium citrate. We extended these observations in experiments on the turtle heart and carried out additional tests on the frog heart with various concentrations of sodium citrate. Depression occurred in both when perfused with citrate, but its action was greater on the frog heart. Contractions of the frog heart were abolished, when it was perfused for ten seconds with a concentrations of $m/200$ sodium citrate. If the turtle heart was perfused with the same solution for five minutes, heart action became weaker and less frequent, but the contractions were still quite vigorous (fig. 4). The difference in resistance to citrate was even more striking, when the perfusion time for the turtle heart was still shorter. Heart action was very moderately decreased in force and no observable difference could be noticed in frequency. In experiments with weaker solutions the contractions were not abolished in the frog heart when perfused with $m/1000$ sodium citrate for about ten seconds. The amplitude was decreased however approximately 25 per cent. If the perfusion time lasted one minute, the amplitude showed a decrease of about 75 per cent. But the same concentration of citrate had no effect on the turtle heart, although in one experiment it was perfused for twenty minutes.

Vagus mechanism

With the vagi intact a distinct slowing of the heart and a typical vagus pulse occurred after the injection of various amounts of citrate into dogs and rabbits. But if citrate was injected after double vagotomy, no appreciable change in the rate of the heart could be noticed. In one experiment only was some retardation of the pulse observed. Stimulation of the cardio-inhibitory cen-

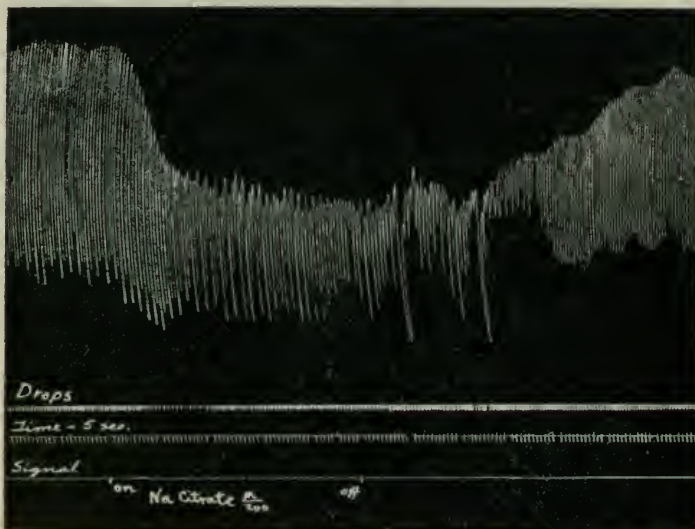


FIG. 4. ISOLATED TURTLE HEART PERFUSED WITH M/200 SODIUM CITRATE

Showing depression and irregularity when sodium citrate was perfused for five minutes. Almost complete recovery when the citrate was washed out by Ringer's. Reduced one-half.

ter in the medulla is produced, therefore, by sodium citrate. Larger doses, however, produced the opposite effect, as the vagus pulse disappeared, and heart action became rapid. We also obtained evidence indicating that citrate acted on the muscle substance of the heart, for when it was injected into atropinized animals the usual fall of blood pressure occurred. In experiments in which heart action was recorded by a Cushny myocardiograph, the effect of citrate was found to be practically the same before

and after atropine. There was a considerable decrease in amplitude in both cases, but after atropine the rate of the heart was not appreciably changed by citrate.

DISCUSSION

The results presented in this communication show that sodium citrate may be both a stimulant and a depressant. This was observed in the effect of citrate on respiration as well as on the circulation when it was given under different conditions. Respiration, it may be recalled, was stimulated in all animals under urethane anesthesia, when the dose was not excessive. But moderate amounts of the salt were without effect or even depressed respiration in dogs under chloretone or morphine anesthesia, thus suggesting that stimulation occurs only when the respiratory center is not depressed. The tendency to slowing of respiration observed when citrate was injected after division of the vagi is of interest in this connection. Perhaps by eliminating an important path of afferent impulses some change in the reaction of the respiratory center to chemical stimuli may take place.

Attention was called to the rise in blood pressure which occurred when citrate was injected into animals with the chest open, while the heart was still considerably depressed. We may add that circulation was but slightly depressed by citrate in dogs with unopened chest, whose blood pressure was low before the injection. These observations, we believe, are of considerable significance. It is quite possible that the low blood pressure produced by the injury to the heart increases the irritability of the vasomotor mechanism. Hence, as in the case of the respiratory center, the central vasomotor mechanism responds to citrate only when in a condition of normal irritability.

SUMMARY

1. Small and medium doses of sodium citrate stimulated respiration in dogs, cats and rabbits under urethane anesthesia.
2. Such doses may cause stimulation or depression of respiration in dogs anesthetized with chloretone.

3. Small and medium doses given to dogs, when morphine-ether narcosis was employed, were without any effect on respiration in some experiments, while in others the action was the same as under chloretone anesthesia.

4. Respiration was depressed or paralyzed by large doses of citrate even when injected slowly into the circulation, and by medium doses when repeated at short intervals.

5. Transitory acceleration of respiration occurred when large doses of citrate were injected intramuscularly in unanesthetized animals; also when injected subcutaneously into decerebrated and into unanesthetized animals.

6. Respiration was only slightly accelerated or was slowed by citrate given after double vagotomy.

7. Small and medium doses of citrate were without effect or produced only a slight fall of blood pressure in dogs under chloretone or urethane anesthesia. A fall or a rise of blood pressure may occur when citrate is given to dogs under morphine-ether narcosis.

8. Small and medium doses of citrate given intravenously to cats under urethane anesthesia produced a fall of blood pressure of 30 to 60 per cent.

9. The fall of blood pressure in rabbits after small and medium doses of citrate was not pronounced, but was considerably increased when the dose was repeated a number of times or when a single large dose was given.

10. After intramuscular injection of large doses of citrate the fall of blood pressure was gradual.

11. The isolated heart of the frog and the turtle was depressed by the perfusion with low concentrations of sodium citrate. The turtle heart was much more resistant to the action of citrate than the frog heart.

12. The mammalian heart in situ was depressed by small and medium doses of citrate even after the vagus endings were paralyzed by atropine.

13. Stimulation of the cardio-inhibitory center in the medulla occurred after small and medium doses of citrate, and depression after large doses.

It gives us pleasure to acknowledge our indebtedness to Dr. Lewis H. Wright of this department for valuable assistance rendered in this investigation.

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EDITED BY

JOHN J. ABEL

The Johns Hopkins University

AND

ARTHUR R. CUSHNY

University of Edinburgh

IN ASSOCIATION WITH

H. G. BARBOUR,
McGill University

J. T. CASH,
University of Aberdeen

A. J. CLARK,
University of London

H. H. DALE,
London

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G. N. STEWART,
Western Reserve University

R. STOCKMAN,
University of Glasgow

CARL VOEGTLIN,
U. S. Public Health and Marine Hospital
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G. B. WALLACE,
University and Bellevue Hospital Medical
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STUDIES ON THE PHARMACOLOGY OF CADMIUM AND ZINC WITH PARTICULAR REFERENCE TO EMESIS¹

ERICH W. SCHWARTZE AND CARL L. ALSBERG

*Pharmacological Laboratory, Bureau of Chemistry, United States Department of
Agriculture, Washington, D. C.*

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PURPOSE OF INVESTIGATION

The experiments herein reported were inaugurated for the purpose of ascertaining the toxicity of cadmium when fed in small amounts for varying lengths of time. This question arose from the suggestion of the use of cadmium solder on food containers in the place of tin solder, and the possible contamination of the food contained therein with cadmium. As the experiments progressed the phenomenon of local emesis, which has heretofore not received a pharmacological investigation as to the nature of the emetic dosage and which is of much medico-legal importance, received special attention. Zinc, which is a well known local emetic substance, was studied for purposes of comparison. Although the authors are not concerned with the industrial alcohol question, it was learned that both cadmium and zinc have been used as denaturants for alcohol (4), for which reason pharmacological information concerning the action of local emetics is at present of immediate practical importance.

PREVIOUS INVESTIGATIONS

The literature on the subject of cadmium is not very extensive. The most complete and the oldest work is that of Marmé (6)

¹ Preliminary results were reported at the tenth annual meeting of the Society for Pharmacology and Experimental Therapeutics, Baltimore, April 24-26, 1919. For proceedings see *Jour. Pharmacol. and Exp. Therap.*, 1919, xiii, p. 504.

which, however, is only very briefly reported. Van Hasselt (11) cites the case of Burdach who produced nausea and vomiting in himself with one-half grain (33 mgm.) of cadmium sulphate. Wheeler (15) reported two cases of cadmium bromide poisoning in man from comparatively large doses (250 to 1000 mgm.) Severi (9) studied the nephritis. Powers (8), using the gold fish, found that some low concentrations of cadmium chloride were actually more toxic than some stronger concentrations. Some investigators, have worked with both cadmium and zinc. The toxicity of these metals to malt diastase (5) and to frogs and dogs under certain arbitrary conditions of experimentation (1) (2) was found in each type of experiment, comparatively speaking, not very different. To man when ingested by mouth (12) and to eggs of *Fundulus heretoclitus* (7) cadmium, however, appears to be considerably more toxic than zinc.

Hayhurst (3) and von Tracinski (13) call attention to the presence of cadmium in zinc ores and to the production of symptoms similar to those of zinc ague when cadmium is inhaled in the form of hot fumes. Stephens (10) reports several instances of chronic poisoning in which amounts of cadmium considerably in excess of the amounts of zinc present have been found in the liver of man.

MATERIALS USED

In the experiments with cadmium, the chloride of this metal was used. It gave no appreciable test for arsenic other than a trace which could have been due to the reagents used. A trace of zinc was present, but no other impurities. Zinc was used in the form of the sulphate, of which more or less must have become converted into zinc chloride because of the available chlorine ions in the animal body or in the food, and also because in the solutions containing 1 per cent or less of zinc some sodium chloride was present.

In order to make the experiments readily comparable in terms of the potent elements contained in the individual salts, all data have been based on the weight of metallic cadmium or zinc.

The standardized solutions of cadmium chloride and zinc sulphate, the arsenic tests and the chemical analyses of viscera were made by Mr. W. D. Collins, formerly of this Bureau, whom we desire to thank for his coöperation.

LOCAL ACTION OF CADMIUM AND ZINC

Effect of cadmium on the unbroken skin. Various alcoholic solutions of cadmium chloride were rubbed on a hairy portion of the forearm. An undershirt which contained 50 mgm. of cadmium (as cadmium chloride) distributed over its entire surface was worn interruptedly for a period of forty hours. No effects were noted and no cadmium was found in the total urine for sixty hours.

Effect of cadmium and zinc on protein. Cadmium and zinc salts, particularly the zinc, when applied to red meat, cause a visible whitening, presumably a precipitation of the protein. A whitening also occurs when the salts of these metals are injected subcutaneously. The necrotic mass is surrounded by a zone of inflammation, which is revealed on the skin by the appearance of a circle or border of ulcerating tissue. Healing follows, with permanent local contraction.

Taste. Both cadmium and zinc possess for man an astringent taste. Meat extract broth and fish juice were less acceptable to the test animals than meat containing these metals, although the liquid diet contained relatively less cadmium or zinc.

Local action of cadmium and zinc on mucous membranes. The post-mortem examination of a rabbit dead from the oral administration of cadmium shows a deep redness of the peritoneal surface of the stomach and the small intestine. With zinc, however, there is a marked whitening, particularly of the duodenum. This whitening is due to the visibility, through the wall of the intestine, of the precipitate on the internal aspect. Nevertheless, some slight evidence of whitening is present in the case of cadmium and also some reddening in the case of zinc. Gross examination of the stomach and intestine shows more ulceration and erosion in the case of cadmium. In the case of zinc there

was presumably a better opportunity for protection of the deeper structures and for concealment of the lesions by the extensive protein precipitation. More mucus was present in the stomachs of the rats which succumbed to cadmium than in the stomachs of those succumbing to zinc. This may have been due to the failure of cadmium to precipitate the mucus in the stomach. In the stomachs of many rats and rabbits which presumably would have survived had they not been killed for post-mortem examination, the duodenal aspect of the pyloric valve was the last part to lose evidence of the protein precipitation.

The local action of cadmium and zinc on diseased tissue was not studied. It should be borne in mind, however, that these substances themselves cause inflammation of intact mucous membranes and that fatalities in test animals result from their absorption, probably at the very site where they cause inflammation.

VOMITING

Cadmium and zinc are local emetics. They also produce vomiting but with considerable general intoxication when administered intravenously in very small amounts. If the entrance into the systemic circulation were a *sine qua non* for emesis when these metals are administered orally, marked intoxication should also occur simultaneously with the emesis from feeding. Moreover, some of the animals which received these metals orally were able to eat a meal of meat free from cadmium rather soon after vomiting.

Cats were used for vomiting experiments. No selection of animals was made except that which was necessary because of the refusal of some to eat. The cadmium or zinc was fed in lean, fresh, hashed meat, in fish juice, and in meat extract solutions. A few of the experiments with cadmium were made on the same animal on consecutive days. Usually they were performed at intervals of about a week.

Cadmium

Emesis from cadmium administered in meat diet. Table 1 gives the results of the emesis tests with cadmium in raw lean meat. When 25 grams or more of food are consumed by a kitten and 50 grams or more by a cat, the minimum emetic concentration of cadmium is 150 parts per million in the diet. The practically certain emetic concentration is 350 to 400 parts per million. From 200 to 250 parts per million is an average (mean) emetic dose.

There was, from time to time, some variation in the reactivity of the same animal, as well as in that of different animals. The time of vomiting also varied and was usually within one to four hours. In general, the emesis tended to occur sooner when the consumption of the food was large or when the concentration of cadmium was high. Vomiting appeared to be quite complete as judged by the bulk or weight of the vomitus, but depended somewhat upon the time of emesis. Emesis from very small amounts of food was rather uncertain. Some cats also showed a disposition to nibble their food, apparently because the emetic action was avoided by regulating the intake. This type of phenomenon must be clearly differentiated from a true habituation.

Other effects. A retained cadmium meal usually affected the sharpness of the appetite the next day. Although some cats began to eat rapidly at first when a meal was offered, they consumed only a part of their food containing the cadmium. Some of these particular animals vomited while others did not. These observations indicate that cadmium probably produced an immediate effect on the stomach, when it did not cause vomiting.

Zinc

Emesis from zinc administered in meat diet. The efficient emetic concentration is 3000 parts of zinc per million (table 1). The effect upon the probability of vomiting of small meals containing 2500 parts of zinc per million was tested upon 11 adult cats. The frequency of vomiting was less than for the average-size

meal of 100 grams. Great difficulty was encountered in inducing cats to eat food containing larger concentrations of zinc, presumably because the meat was decidedly hardened or "pickled." For that reason insufficient data were obtained for large meals. With a concentration of 1500 parts per million the cats employed ate large meals, but the frequency of vomiting was

TABLE 1
Vomiting experiments using a medium of raw hashed lean meat

PARTS PER MILLION IN FOOD	TOTAL EXPERIMENTS	EXPERIMENTS WITH NO VOMITING	EXPERIMENTS WITH VOMITING	PERCENTAGE OF EMETIC EFFICIENCY
Cadmium				
100	6	6	0	0
150	41	31	10	24
200	33	16	17	51
250	37	17	20	54
300†	33	14	19	57
350†	5	1	4	80
400†	21	1	20*	96
Zinc				
1000	6	6	0	0
1500	24	20‡	4	17
2000	10	5	5	50
2500	18	5	13	72
2500	11	8§	3	27§
3000	5	0**	5**	100

* 15 cats ate from 50 to 110 grams. 5 others from 150 to 200 grams.

† Arithmetical mean for 5 largest doses given, which were non-emetic, is 330 parts per million.

‡ Including 6 kittens eating 20 to 100 grams. Four cats ate large meals of 150 to 200 grams meat.

§ Half rations (50 grams meat) to grown cats. Shows the effect of amount of food upon the probability of vomiting.

** 20 to 100 grams consumed.

not increased significantly over that caused by meals of ordinary size containing this concentration.

Other effects. One animal tried to vomit but did not succeed. This was taken as evidence of nausea. After retaining zinc meals some animals refused additional food on the same as well as on the following day.

THE NATURE OF THE EMETIC DOSAGE

As already stated the emesis results have been recorded on the basis of the concentration of cadmium in the diet. Since it seemed eminently desirable in some instances to substitute such a procedure for the universal practice of expressing toxicity or emetic potency of poisonous substances in terms of absolute amounts, the question arose: Is such a procedure justified? The first information bearing upon this point was obtained in the continued feeding experiments. Several cats which declined in weight on certain absolute amounts of cadmium recovered when the quantity of food offered was doubled, the absolute amount of cadmium remaining the same. This is precisely what might be expected if the emesis is in the main purely a local effect due to the concentration of cadmium in the diet.

Method. The method selected for studying this question was that of analyzing the results both upon the basis of the concentration of cadmium in the diet and upon the basis of milligrams of cadmium per kilo body weight and ascertaining which method gave the most concordant results. Since it was obvious that either method of computation would give equally uniform data when the size of the experimental animals and the amount of food were fairly constant, it was necessary to vary one or both of these factors.

Cats of different sizes were therefore selected because smaller animals have relatively greater food requirements and larger stomachs than larger animals. These animals were fed amounts of food ranging from what would be considered liberal down to the minimum sufficient for maintenance. The cats varied about 600 per cent in weight and, as judged by the size of a satisfying meal, about 300 per cent in stomach capacity.

ANALYSIS OF EXPERIMENTAL RESULTS

Table 2 gives data showing the relation of the minimum emetic doses and the maximum sub-emetic (retained) doses of cadmium and zinc, calculated on the basis of milligrams per kilo of body weight. It is readily seen that the cats are segregated into

groups according to sizes. Only kittens are to be found among the most resistant and only grown cats among the least resistant animals. This can be explained on the basis that the dosage is not dependent upon the milligrams per kilo ingested, but upon the concentration of emetic substance in the food, since, by the latter method of computation of the results, there is no segregation into classes. The popular opinion that in kittens idiopathic "fits" and emesis are more common than in grown cats does not

TABLE 2

Lowest emetic and highest non-emetic doses on the basis of milligrams per kilo of body weight of cats

LOWEST EMETIC DOSES			HIGHEST NON-EMETIC DOSES		
Body weight, grams	Emetic substance consumed		Body weight, grams	Emetic substance consumed	
	Milligrams	Milligrams per kilo		Milligrams	Milligrams per kilo
Cadmium					
2260	15	5.54	1320	30	22.7
3650	15	4.11	675	14	20.8
2450	15	6.12	1300	25	19.2
2980	18	6.04	545	10.75	19.7
2000	10	5.00	1010	20	19.8
Average.		5.36			20.4
Zinc					
3850	125	32.4			
2450	60	24.5	790	125	158.0
2100	125	59.5	890	125	140.3
2250	125	51.1	790	150	189.8
Average.		41.9			162.7

enter into the consideration of the data here reported, since it developed by one method of computing results that the kittens used appeared to be the more resistant, in contrast to this popular idea.

In table 3, data taken from tables 1 and 2 are compared. The range between the minimum emetic and the maximum retained dose is much less when the computation is made upon the

basis of the concentration of the emetic substance in the food. If the emesis is a function of the concentration, it would be expected that computation of the emetic dosage on the basis of body weight for cats of varying sizes would give more varying results.

TABLE 3

A comparison of the two methods of computing the emetic dosages based upon the data used in the preceding tables

OBSERVATION	PARTS PER MILLION IN FOOD BASIS			MILLIGRAM PER KILO BASIS			RATIO OF RANGES OBTAINED BY THE TWO METHODS
	Minimum emetic dose	Maximum retained dose	Range (multiples of lesser)	Minimum emetic dose	Maximum retained dose	Range (multiples of lesser)	
Cadmium							
Single.....	150	400	1.7	4.11	22.7	4.5	1:2.65
Average.....	150*	330*	1.2	5.36*	20.4*	3.0	1:2.5
Zinc							
Single.....	1500	2500	0.67	24.5	189.8	6.7	1:10.0
Average.....	1500*	2500*	0.67	41.9†	162.7‡	2.9	1:4.3

* Average of five observations.

† Average of four observations.

‡ Average of three observations.

INFLUENCE OF MEDIUM IN WHICH EMETIC SUBSTANCE IS ADMINISTERED

There are given in table 4 the results of experiments with cadmium and zinc administered in liquid diet (fish juice and meat extract solution). The vomiting occurred sooner from smaller amounts of food and from a lower concentration of the metals than with solid food, such as raw meat. More difficulty was encountered in getting cats to consume the liquid than the meat. These observations upon the effectiveness of liquid media are explainable on the basis that the degree of protein precipitation was relatively less in the liquid and that the mucous membrane of the mouth and stomach had a better opportunity to come in contact with the administered substance. The failure of emesis in certain of the experiments, especially with cadmium,

can be explained by the more rapid passage out of the stomach of the small amount of liquid contents. The possibility also exists that the large number of instances in which the liquid food was rejected decreased the percentage of emesis through the elimination of many of the more sensitive cats. Cadmium and zinc are each two to three times more potent in the liquid media used than in a solid medium of lean, hashed meat.

TABLE 4
Vomiting experiments with liquid food

PARTS OF METAL PER MILLION OF LIQUID	TOTAL EXPERIMENTS	EXPERIMENTS NOT RESULTING IN VOMITING	EXPERIMENTS RESULTING IN VOMITING	PERCENTAGE EMETIC EFFICIENCY
Cadmium				
50	5	5	0	0
100	13	2*	11†	85
150	9	2	7†	78
200	3	1†	2	67
250	2	1‡	1	50
Zinc				
1000	15	8†	7	53
1500	16	11*	5§	69

* Two cats ate 15 cc. each.

† One cat ate 15 cc.

‡ Consumed 20 cc.

§ Three cats ate 15 cc. each.

RELATION OF RESULTS OF VOMITING EXPERIMENTS TO CLINICAL OBSERVATIONS

The correlation of the data obtained in the vomiting experiments here reported with the data available for man is not an easy matter, because in therapeutics dosages are stated in terms of absolute amounts and not in terms of concentration. Burdach is stated (11) to have produced vomiting in himself from $\frac{1}{2}$ grain of cadmium sulphate, which is equivalent to approximately 15 mgm. of metallic cadmium. If this dose were contained in from 100 to 150 cc. of liquid and ingested on an empty stomach this report would be in harmony with the results herein

reported. On the basis of the cat experiments, cadmium sulphate should be at least 15 times more efficient than zinc sulphate. One half grain (30 mgm.) of cadmium sulphate is from $\frac{1}{30}$ to $\frac{1}{60}$ of the size of the usual dose of zinc sulphate. If the calculated potency ratio of cadmium sulphate to zinc sulphate in the cat (about 15:1) will hold for man, it seems probable that Burdach took the dose of cadmium in a very small amount of food or liquid. The data for cadmium in cats would, therefore, seem to be directly applicable to man.

The therapeutic emetic dose of zinc sulphate (U.S.P.) is given by many authorities as from 1 to 2 grams. The amount of zinc as metal contained in the larger dose is 460 mgm. This amount of zinc if contained in approximately 150 cc. of stomach contents would represent a concentration of 3000 parts of zinc per million. It is possible that this volume could be increased to 300 or even 450 cc. if the stomach contents were fluid and still produce vomiting without the necessity of increading the absolute amount of zinc present. The uncertainty of zinc as an emetic in man under the prevailing therapeutic usage is very probably due to the insufficient amount administered, and to the varying character and amount of the stomach contents. An increased effectiveness of an emetic because of the possible hypersensitivity of the stomach in case of poisoning or disease is also to be considered. The data for normal cats herein presented can be considered indicative of the concentrations which under similar conditions would be effective for a healthy man.

Significance of emesis experiments. It is believed that it has not been pointed out heretofore by any investigator that the effective emetic dose, in the case of substances acting locally upon the gastric mucosa, can be expressed with an appreciable degree of exactness only in terms of the concentration in the vehicle in which it is exhibited. Such considerations as these herein presented should be taken into account in drawing deductions from animal experiments concerning the behavior of man. In a few cases, such as local anesthetics like cocaine, and astringents like adrenalin, this manner of calculating dosages has long been the custom. If such considerations be kept in mind

many of the apparent discrepancies between experiments with animals and experiences with man can be made to disappear.

Although the authors are not aware that any clinician has called attention to the necessity of calculating the emetic dosage in terms of the concentration, these results are in perfect harmony with clinical custom and experience. Waddell (14) states that dilution and giving after food diminishes the nauseating effect of fluorides. It is universally the custom to administer irritating substances such as arsenic trioxide in the dissolved state, "well diluted" and "after meals," both of which conditions decrease the irritation.

SYSTEMIC ACTION OF CADMIUM AND ZINC

A large number of experiments were performed in which both cadmium and zinc salts were administered to animals intravenously, subcutaneously and orally. The results of these experiments have been summarized in table 5. It will be seen by comparing the data in this table with those concerning emesis (tables 1 and 4) that the relative toxicity of cadmium to zinc is not as great as the relative emetic potency. The rabbits which were fed on a diet consisting entirely of carrots died more acutely and from smaller doses of cadmium than those on an oat and carrot diet, although when they lived they withstood the nephritis better. The lethal dose for rabbits on either of these diets was practically the same. A blue discoloration of the testicle in the rat, both ante- and post-mortem, was observed in most of the cadmium experiments.

The special features in each type of experiment are as follows:

Intravenous administration. The ratio of the toxicity of cadmium to zinc for one species does not necessarily exactly hold for any other species. Cadmium appears to be relatively less toxic to the cat and the dog than to the rat and rabbit, when judged by zinc as a standard. This phenomenon is due chiefly to the greater resistance of the cat and dog for cadmium. Both cadmium and zinc cause vomiting and diarrhea when administered intravenously. Inflammation of the stomach and intestine is found upon post-mortem examination. Death from these

TABLE 5

Toxicity of cadmium and zinc

ANIMALS USED	METAL USED	INTRAVENOUS ADMINISTRATION			SUBCUTANEOUS ADMINISTRATION			ORAL ADMINISTRATION		
		Usually sublethal	Inter-mediate	Usually lethal	Usually sublethal	Inter-mediate	Usually lethal	Usually sublethal	Inter-mediate	Usually lethal
Rats	{ Cadmium Zinc	mgm. per kilo 1.0-1.5	mgm. per kilo 2.0	mgm. per kilo 3.0	mgm. per kilo 10-25	mgm. per kilo 40	mgm. per kilo 100*	mgm. per kilo 250*	mgm. per kilo 150*	mgm. per kilo 500*
		5.0-7.5	10.0	11.2-14.0	75-150			{ 50† —	{ — 100‡	{ 70† 150‡
Rabbits	{ Cadmium Zinc	1.35	1.5-1.7	2.0	10-20*	25*	50*			
			6.5	10.0	50-100			250†	350†	435-500‡
Cats	{ Cadmium Zinc	2.5	3.0-4.0	5.0*	10	20*	25-40			
		6.5	10.0			100-115*				
Dogs	{ Cadmium Zinc	3.0-4.0*	10.0*	5.0*						
				15.0-25.0*						

* Data probably insufficient.

† 0.1 and 0.2 per cent solutions of Cd used.

‡ 1 per cent solution of Cd used.

metals is probably due to respiratory paralysis, although a fibrillation of the heart, which can be observed if post-mortem examination is performed soon enough, is to be considered.

Subcutaneous administration. Since neither cadmium nor zinc were absorbed well from the subcutaneous tissue, the method is not suitable for accurate work. When rabbits were injected on the side of the spine, an edema occurred on the ventral abdominal wall.

Oral administration. Cadmium was more toxic in the dilute solutions tried than in the concentrated solutions. Since the lethal dose of zinc was much larger than that of cadmium, it was impossible to administer the zinc in sufficiently dilute solution to ascertain its behavior under comparable circumstances. Moreover, zinc is the better protein precipitant, another factor rendering control of the experiments difficult.

FEEDING EXPERIMENTS WITH CADMIUM

A series of cats, totaling 17 in number, were fed with amounts of cadmium ranging from 5 to 100 mgm. per day. The body weight of these animals was not taken into account in assigning the amounts to be fed. Unless otherwise noted the cats received 100 grams of meat daily for five days of the week. On Saturdays they were fed 150 grams, containing the same absolute amount of cadmium as were received on each of the other five days. No deductions were made for food which was vomited. The uneaten food was weighed, but no allowances were made for evaporation of moisture from the meat. The animals were weighed weekly and their general condition was noted.

The results of 11 of these experiments, which were started in August, 1918, and continued for varying lengths of time until October, 1919, are given in table 6. The results of these experiments are divisible into two groups,—those in which 200 parts or less of cadmium per million parts of food were fed and those in which 250 parts or more of cadmium were fed. Several cats belong to both groups by virtue of the fact that when either the amount of food or the absolute amount of cadmium was increased, the concentration of cadmium was accordingly changed.

On the whole, animals on a diet containing 200 parts per million of cadmium maintained their own or increased in weight. Occasionally vomiting occurred or part of the food containing cadmium was uneaten. The character of the appetite recorded in table 6 refers only to the ability to eat within 24 hours. Cats receiving 250 parts or more per million usually vomited or left much of their food and lost in weight. In addition to the data given in table 6, 6 other cats were used, upon which a total of 17 unsuccessful attempts were made at various times to inaugurate continued feeding experiments with amounts of cadmium ranging from 250 to 1000 parts per million. These experiments were discontinued because of the refusal of the food. When the concentration of cadmium was reduced to or below 200 parts per million, or when no cadmium was fed, recovery was usually observed. No symptoms which would indicate either systemic cumulative effect or an habituation to this metal were observed.

There were two epidemics of distemper. One occurred in the winter, apparently because the laboratory was not well heated at night. In the other, which occurred in the summer, the cadmium was probably a predisposing factor. The chief effect of this disease was to lessen the appetite. It was found necessary to take most of the animals off the cadmium diet, since they did not recover from the symptoms of the disease, such as sneezing, coughing, and loss of appetite, until this was done.

The data obtained from these experiments harmonize remarkably well with those from the vomiting experiments. The mean emetic concentrations are 200 to 250 parts of cadmium per million in the diet. As would be expected, vomiting and decreased appetite would result in loss of weight, which, if continued, would result in death.

This, however, is not the only criterion by which to judge the deleterious action. Although cats receiving 200 parts and less per million only vomited occasionally, they usually did not eat their food as readily as a normal cat eats a meal. Nibbling was constantly observed in the experiments. This was apparently the means by which the cats receiving the larger emetic concentrations were able to retain a little food and thereby prevent acute

TABLE 6
Continued feeding experiments with cadmium; data by consecutive periods showing significant phenomena

EXPERIMENT NUMBER	CONCENTRATION OF CADMIUM IN DIET	MEAT OFFERED DAILY	DURATION OF PERIOD	FINAL BODY WEIGHT	GAIN OR LOSS	ABILITY TO EAT ALL FOOD* OFFERED	TIMES VOMITED	REMARKS
		grams	weeks	grams	grams			
1	0	100	0	2340		Good		
	50	100	21	2430	+ 90	Good	1	Killed. Autopsy normal
2	0	100	0	760		Good		
	50	100	22	1760	+1000	Good	1	Grew fairly well
	50	100	3	1440	- 320	Fair	0	Contracted distemper
	0	100	4	1440	0	Good	0	
	0	150	3	1630	+ 190	Good	0	
	0	100	2	1590	- 40	Good	0	Recovered
	50	100	9	1750	+ 160	Good	0	
	50	100	2	1200	- 550	None	0	Died of distemper
	0	100	0	2590		Good		
	100	100	5	2020	- 570	Good	0	Parturient
3	67	150	2	2000	- 20	Good	2	
	0	150	3	1970	- 30	Good	0	2 kittens weaned
	67	150	21	2870	+ 900	Good	0	Slight cough; recovered
	100	100	3	2770	- 100	Good	0	
	300	100	10	2200	- 570	Fair	4	Declined
	300	100	2	1850	- 350	Poor	0	Contracted distemper
	0	100	19	2390	+ 540	Good		Recovered; killed

4	0	100	0	2450	+ 600	Good	2	No distemper
	150	100	34	3050	- 800	Good	6	Cadmium increased
	300	100	9	2250	- 160	Fair	0	Contracted distemper
	300	100	2	2090	+ 980	Fair		Recovered; killed
5	0	100	19	3070		Good		
	0	100	0	2760		Good	3	Nibbled food
	200	100	16	2750	- 10	Good	2	Contracted distemper
	200	100	5	2000	- 750	Fair	0	Recovered
6	0	150	6	2530	+ 530	Good	0	
	200	100	16	2400	- 130	Good	0	
	200	100	2	1730	- 670	Poor	0	Contracted distemper
	0	100	19	2820	+1090	Good		Recovered; killed
7	0	100	0	2505		Good		
	250	100	2	?	Loss	Poor	?	Vomited and starved; died
8	0	100	0	1295		Good		
	300	100	6	880	- 415	Poor	4	Died of inanition
8	0	100	0	1380		Good		
	300	100	7	1120	- 260	Poor	12	Declined
	150	200	13	2410	+1290	Good	1	Recovered
	300	100	4	1950	- 460	Poor	0	Began coughing 22nd week
	150	200	2	1780	- 170	Poor	0	Distemper continued
	0	150	8	1900	+ 120	Good	0	Recovered
	300	100	8	1770	- 130	Fair	0	Nibbled food
	300	100	2	1180	- 590	Poor	0	Died of distemper

TABLE 6—Continued

EXPERIMENT NUMBER	CONCEN- TRATION OF CADMIUM IN DIET	MEAT OFFERED DAILY	DURATION OF PERIOD	FINAL BODY WEIGHT	GAIN OR LOSS	ABILITY TO EAT ALL FOOD OFFERED	TIMES VOMITED	REMARKS
9	parts per million	grams	weeks	grams	grams			
	0	100	0	2405		Good		
	350	100	7	1530	- 875	Poor	8	Declined
10	175	200	14	2570	+1040	Good	0	Recovered; killed
	0	100	0	2285		Good		
	350	100	7	1650	- 635	Poor	4+	Declined
	175	200	13	2710	+1060	Good	2	Recovered
	350	100	14	1505	-1205	Poor	1	Declined
	0		5	1960	+ 455	Good	0	Recovered
	350	100	3	1640	- 320	Fair	0	Declined
	350	100	2	1450	- 190	Poor	0	Contracted distemper
	0	100	19	2020	+ 570	Good	0	Recovered; killed
	0	100	0	2290		Good		
11	500	100	7	1270	-1020	Poor	2+	Declined
	250	200	4	1395	+ 125	Fair	0	No recovery; killed

inanimation. The immediate cause of the nibbling in some instances might have been the taste. It was observed, however, in the series of vomiting experiments that a single dose of cadmium at times depressed the appetite on the same day, or even the next day, for meat containing no cadmium, whether vomiting had been produced or not. The smallest amount of, or the least

TABLE 7

Cadmium found in viscera of cats used in continued feeding experiments

CAT NUM- BER	DURA- TION OF EXPER- IMENT	CADMIUM OFFERED DAILY*	LIVER			KIDNEY			SPLEEN		
			Weight	Cadmium found†		Weight	Cadmium found†		Weight	Cadmium found†	
	weeks	mgm.	grams	mgm.	per cent	grams	mgm.	per cent	grams	mgm.	per cent
1	21	5	88.0	0.4	0.0005	11.5	3.8	0.0230	4.0	0.5	0.0012
2§	45	5	50.8	1.9	0.0037	17.8	1.3	0.0073	3.2	0	0
3	64	10 to 30	83.0	3.5	0.0042	27.5	2.9	0.0105			
4	64	15 to 30	80.0	11.2‡	0.0140						
5	64	20	102.0	1.8‡	0.0018	31.0	4.0	0.0129			
7	6	30	29.2	1.6	0.0055	12.8	2.7	0.0211	4.2	0.9	0.0214
8	44	30	46.65	38.4	0.0823	16.0	9.3	0.0581	2.15	0	0
9**	21	35	125.0	4.0	0.0032				6.5	0	0
10††	63	35	60.0	15.6	0.0260	20.5	6.4	0.0312	6.5	0	0
11‡‡	11	50	81.5	1.4	0.0017	17.1	4.5	0.0263	2.0	0	0

* Offered but not necessarily consumed or retained.

† Mr. W. D. Collins, the analyst, states that several tenths of a milligram may be missed.

‡ Part of sample lost in analysis.

§ Femur, stomach and intestines, muscle, lungs and heart contained no cadmium.

** Brain skin and blood contained no cadmium.

†† Brain and bone contained no cadmium.

‡‡ Blood, bile and gall bladder, muscle, femur and large intestine wall contained no cadmium.

concentration of, cadmium in the diet which might produce definite but transient disturbances in the stomach or intestines cannot be stated from these experiments. It appears that with suitable conditions for more detailed observation, experiments on the cat may prove extremely useful in studying functional disturbances which do not lead to such profound changes as are herein reported.

Table 7 gives the results of the chemical analyses of the viscera of the cats fed cadmium for extended periods. The quantitative interpretation of these few data is open to difficulties because of variations in the amount of cadmium retained and because of the absence of analyses of control animals. The liver, kidneys, and spleen were the only organs found to contain detectable and weighable amounts of cadmium. Sometimes the spleen contained none. Considering its size, the kidney generally contained relatively much more cadmium than the liver. In general, there seems to be a tendency for more cadmium to be stored up the larger the dose and the longer the experiment. This storage is not so great but it may be the expression merely of the fact that the cadmium is absorbed more rapidly than it is excreted. After-periods on a cadmium-free diet seem to be associated with a loss of stored cadmium. Cadmium was found in urine collected from the cages. This, together with the fact that relatively more was found in the kidney, marks this organ as a factor in the elimination. It is to be noted, however, that the percentage of cadmium found in the cat livers is in general agreement with that reported in human livers (10), in which cases chronic cadmium intoxication was suspected.

SUMMARY

On intravenous injection protein precipitation may occur intravascularly. This phenomenon must be considered in the interpretation of such experimental data. Zinc, calculated as the metal, is about equally toxic for all species studied (rats, rabbits, cats and dogs). Cadmium is about three times more toxic to cats and dogs, but to rabbits and rats four or five times more toxic, than zinc.

Subcutaneous administration furnished little information of interest because of the protein precipitation at the site of injection and poor absorption.

On oral administration the lethal dose of zinc is from five to seven times greater than that of cadmium. In dilute solutions used, cadmium is more toxic than in more concentrated solutions.

In the case of animals that vomit, such as cats, the chief effect of the oral administration of cadmium and zinc salts is emesis, which is dependent upon the concentration in which these substances are present in the gastric contents. This local effect varies with the character of the food, being more powerful when the metals are administered in diets of liquids than when administered in those of raw hashed, lean meat. The consumption of an average-sized meal of raw hashed meat containing 350 to 400 parts per million of cadmium or 3000 parts per million of zinc is almost always followed by emesis. Cadmium calculated as the metal is eight to nine times more effective as an emetic than zinc.

The results of continued cadmium feeding experiments agree remarkably well with the data obtained in the vomiting experiments. Concentrations of cadmium of 250 or more parts per million in the diet were incompatible with life. This is also the mean emetic concentration. Recovery followed reduction of this concentration. No evidence of cumulative systemic action was obtained in these experiments. However, slight loss of appetite, nibbling of food and occasional vomiting indicate that, although apparently compatible with life, the presence in the food of the lower concentrations tried is decidedly objectionable.

No evidence of storage of cadmium other than in the kidney, liver and spleen was obtained. The kidney usually contained the most metal relatively, while the spleen contained the least and sometimes none.

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THE CARBON MONOXIDE CONTENT OF TOBACCO SMOKE AND ITS ABSORPTION ON INHALATION

J. PERCY BAUMBERGER¹

Contribution from the Laboratory of Physiology, Stanford University

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This paper is the first of a series on tobacco smoke in connection with two papers on the effect of smoking on industrial fatigue which have already been published (1), (2).

INTRODUCTION

The investigation was undertaken because of the unsatisfactory state of present knowledge of the subject. Several other investigations have already been made on the carbon monoxide content of tobacco smoke but, as is shown in table 1 the conclusions are exceedingly variable. The explanation of the difference in results is probably the unsatisfactory methods used. The iodine pentoxide method here used is generally admitted to be far more accurate (3) than any of the methods used by these authors and since it has never been applied to the analysis of tobacco smoke, we feel amply justified in repeating the work.

METHOD

The method used was the oxidation of carbon monoxide to carbon dioxide by iodine pentoxide, as described in the paper by Weisman (9), the liberated iodine being determined. The reaction is as follows: $I_2O_5 + 5CO = 5CO_2 + 2I$. The iodine is collected in potassium iodide solution and is titrated with

¹ The author wishes to acknowledge his indebtedness to his colleagues Dr. E. G. Martin and Dr. E. C. Franklin for advice in connection with this paper, and to J. R. S. and J. M. B. for acting as subjects.

sodium thio-sulphate with starch as an indicator. The disappearance of the blue color of starch indicating the complete combination of free iodine as follows: $2\text{Na}_2\text{S}_2\text{O}_3 + 2\text{I} = 2\text{NaI} + \text{Na}_2\text{S}_4\text{O}_6$.

As I_2O_5 is decomposed by moisture, organic matter and high temperature as well as CO, the former three factors must be guarded against. The organic matter and moisture were screened out by a series of absorption bulbs and drying tubes. High temperature was guarded against by an oil bath.

Figure 1 shows the apparatus as set up. Smoke was drawn through the apparatus from left to right by means of two aspirator bottles drawing in the direction indicated by the arrow *A*. Puffing of a cigarette (*B*) was produced by turning stop cock (*C*) on and off. The smoke passed over cotton wool (*D*) and then through four modified Liebig Bulbs containing concentrated H_2SO_4 (*E*) fuming H_2SO_4 (*F*) concentrated KOH (*G*), concentrated H_2SO_4 (*H*) and then through four U tubes containing respectively solid KOH (*I*), solid CaO (*J*), cotton (*K*) and glass wool (*L*) and finally over I_2O_5 in glass wool in a U tube (*M*) kept by an oil bath at 180°C . In *M* the I_2 was liberated and passed into the wash bottles (*N*) containing KI + starch where it was absorbed. All connections of the apparatus was made of suction pump rubber tubing sealed with sealing wax. The U tube containing the I_2O_5 was sealed with melted rubber. The apparatus was free from leaks and the unstable portion of I_2O_5 was decomposed by twenty-five hours continuous passage of air through the apparatus and each time an experiment was run the apparatus was flushed out with air for twenty minutes. The iodine liberated was titrated with $\text{N}/1000\text{Na}_2\text{S}_2\text{O}_4 = 0.061 \text{ cc. CO at } 25^\circ\text{--}760 \text{ mm.}$ Soluble starch was used as the indicator. The KI to be used for absorption of I_2 was tested for free iodine.

As it was feared that unsaturated hydrocarbon and moisture might be passing through the screen of absorption bulbs and decomposing the I_2O_5 , at the suggestion of Dr. E. C. Franklin a spiral glass tube in a Dewar flask (*O*) filled with liquid air was inserted between *L* and *M*. The liquid air² would liquefy and

² After using this method I find that it has already been described by Teague (3).

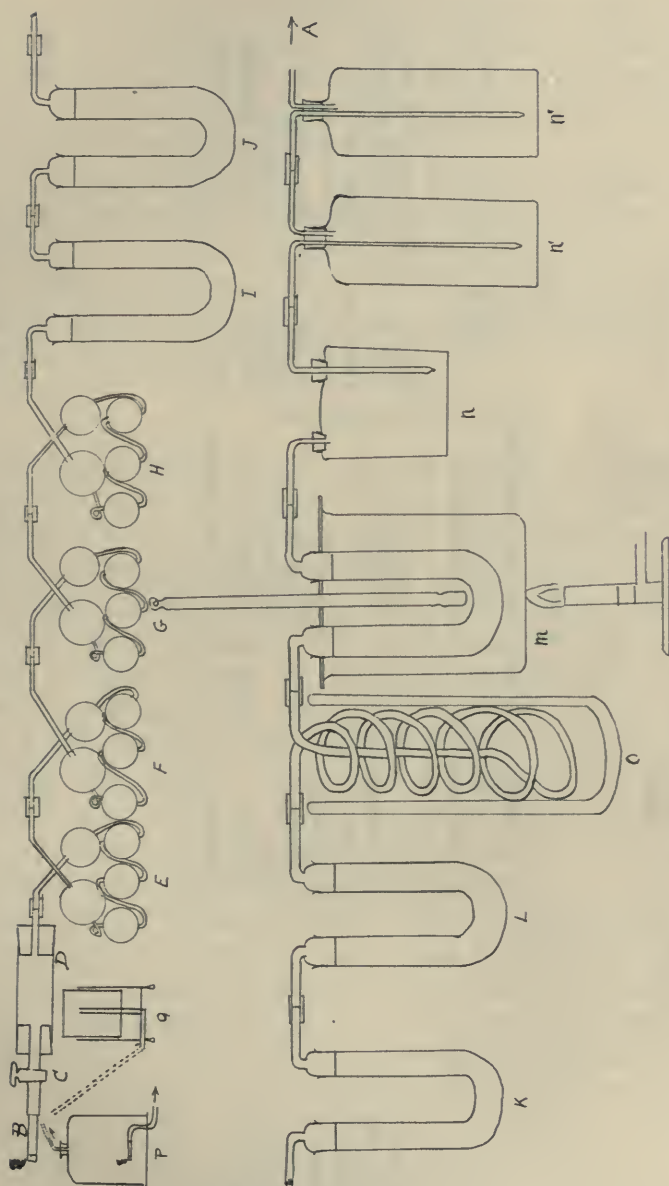


FIG. 1. APPARATUS FOR THE ABSORPTION OF CO IN TOBACCO SMOKE

TABLE 1
Summary of some previous investigations

AUTHOR	TOBACCO	CC. CO/GRAMS TOBACCO	METHOD OF SMOKING	METHOD OF ANALYSIS OF CO
Habermann (4).....	Cigars	14.8	Puffed	Ammoniacal silver
Pontag (5).....	Cigarettes	41.0	Puffed	Palladium chloride
Thoms (6).....	Cigars	0.02	Continuous	Haemoglobin
Lehmann (7).....	Cigarettes	15.0-23.2	Puffed	Not stated
Lehmann.....	Cigars	74.0-85.0	Puffed	Not stated
Lehmann.....	Pipe	74.5-77.8	Puffed	Not stated
Wahl (8).....	Cigar	6.8 cc. CO/100 cc. smoke	Puffed	Cuprous chloride
Wahl.....	Tobacco	2.3 cc. CO/100 cc. smoke	Puffed	Cuprous chloride

cause to remain in the spiral tube, any compounds other than CO capable of decomposing I_2O_5 . The results obtained in the analyses of smoke using this liquid air screen are essentially the same as without it, therefore we conclude that our screen of absorptive bulbs, etc., was adequate.

A qualitative test of the gas entering the U tube containing the I_2O_5 was made by inserting an absorption bottle of hemoglobin solution in place of the liquid air screen. The hemoglobin was tested spectroscopically for CO-hemoglobin with definite positive results showing that CO was passing through the absorption bulbs to the I_2O_5 .

EXPERIMENTS

Our first experiment with the CO content of tobacco smoke was made with "Piedmont" cigarettes. Two cigarettes were smoked at a time using a Y tube. The puffing was done by aspirators as described above. The result of all the experiments are given in table 2. Experiments 37 to 40 with "Piedmonts" gave a yield by weight of CO of 0.19 and 0.31 per cent of the weight of tobacco smoked. Continuous suction gave a similar quantity, 0.25 per cent. A "Glad" cigar was then smoked and gave a yield twice as great (0.59 per cent). The smoke from the burning point of the "Glad" cigar (experiment 43) also yielded CO though in the small quantity of 0.05 per cent. The smoke from the burning point was collected by suction from a bell jar as shown much reduced in size at P in figure 1.

The fact that the smoke puffed from the cigar had a CO content ten times as great as the smoke from the burning point seemed to indicate that the CO was a result of incomplete combustion caused either by drawing CO_2 away from the burning point through a region of hot coals where reduction to CO took place or by drawing the CO away before complete oxidation to CO_2 had occurred. That the rapid withdrawal of the smoke does result in higher CO content is shown in experiment 42 where by forcibly drawing the smoke away from the burning point of a "Glad" cigar, the CO content of the smoke was increased ninefold.

TABLE 2
Analysis of CO content of tobacco smoke

EXPERI- MENT NUM- BER	BRAND	HOW SMOKED	SUB- JECT	GRAMS	PUFFS	SMOKE cc.	CO PER GRAM TOBAC- CO	CO	VOL- UME PER CENT	WEIGHT PER CENT	PARTS CO: 10,000 AIR
37	Piedmont	Machine		2±	103 x 2		cc.	cc.		0.19	
39	Piedmont	Machine		2±	Continuous		1.6	3.3		0.25	
40	Piedmont	Machine		2±	165 x 2		2.2	4.4		0.31	
41	Glad	Machine		1.65	155		2.7	5.4		0.59	
42	Glad	Burning point smoke, strong suction		2.40	80	4250	5.2	8.5	0.22	0.45	22.0
43	Glad	Burning point smoke, weak suction		2.00	165	4250	3.9	9.4	0.021	0.05	2.1
44	Filter paper	Machine		1.29	140	4250	0.4	0.9	0.019	0.07	1.9
45	Filter paper and C ₂ H ₄ O ₂	Machine		0.60	51	1500	0.6	0.8	0.06	0.17	6.0
46	Filter paper and C ₂ H ₄ O ₂	Machine		0.60	69	2500	1.5	0.9	0.368	1.77	36.8
47	Filter paper	Burning point smoke, weak suction		0.74	Continuous	1000	15.3	9.2	0.07	0.11	7.0
53	Fatima	Inhaled	S.		8	6200	0.9	0.7	0.063		6.3
54	Fatima	Puffed	S.		8	6200		3.9	0.25		25.0
55	Fatima	Inhaled	S.	0.285	8	6200	8.1	2.3	0.037	0.91	3.7
56	Fatima	Puffed	S.	0.305	8	6200	14.8	4.5	0.072	1.70	7.2
57	Fatima	Inhaled	B.	0.610	14	3850	7.2	4.4	0.12	0.84	12.0
58	Fatima	Puffed	B.	0.535	14	5500	20.4	11.1	0.20	2.39	20.0

Filter paper was then rolled into the form of cigarettes and the CO content determined. The yield was 0.07 per cent which was lower than tobacco. The yield from the burning point was 0.1 per cent. Dipping the filter paper in acetic acid and then drying resulted in an increased CO output on smoking (experiments 45 and 46). This effect of organic acids on CO yield is well known. It may be that the citric, malic and oxalic acid content of tobacco influences the high CO content of tobacco smoke.

Experiments 53 to 58 were performed on subjects to determine amount of CO retained from inhaling. In experiments 54, 56 and 58, the subjects puffed cigarettes in a normal way and blew out all the smoke into a spirometer. The spirometer was properly balanced so that the smoke could be blown in without effort. The surface of the water in the spirometer was covered with petroleum oil. After all the smoke had been blown in, the volume was read directly from the spirometer and the smoke by pressing gently on the upper cylinder of the spirometer was slowly forced through the CO analysis apparatus. The spirometer is shown much reduced in size in *Q*, figure 1. In experiments 53, 55 and 57, the subjects inhaled the smoke from each puff of a cigarette so that they were able to open the mouth without any smoke coming forth. The nose was then held and the smoke blown into the spirometer. This exhaled smoke was blown into the analysis apparatus in the manner described above. Between each determination, the 8-liter spirometer was flushed out by filling with pure air from a pressure pump and the emptying by an air suction pump, repeating this process five times. Experiment showed that the spirometer then held no more CO.

The analyses show that when smoke is puffed into the mouth and then blown out, the smoke contains CO from 0.072 to 0.25 per cent by volume of air blown out. The percentage volume of CO in exhalations (after inhaling the smoke) is much smaller, namely 0.037 to 0.12 per cent. The variation is probably due to the various strengths of suction in puffing used by different smokers, therefore the experiments were run in pairs on the same subject; thus, experiment 53 should be compared with 54,

55 with 56 and 57 with 58. This comparison is given in table 3 and shows that less CO leaves the mouth after inhaling the smoke, than after merely drawing the smoke into the mouth. This result holds good calculated as cubic centimeter CO recovered per gram tobacco smoked, or as cubic centimeter CO recovered per puff or inhalation. The difference in amount of CO recovered after the smoke was drawn into the mouth and then puffed out as compared with the amount recovered after inhaling the smoke and then exhaling, may be considered as CO retained in the body. The seventh column of table 3 shows the probable number of cubic centimeters of CO absorbed in the body per inhalation to vary from 0.27 to 1.42. In the last column the

TABLE 3
Retention of CO on inhaling tobacco smoke

EXPERIMENT NUMBER	BRAND	HOW SMOKED	SUBJECT	CC. CO/GRAM	CC. CO/PUFF	CC. CO/EXHALATION	PROBABLE CC. CO/INHALATION ABSORBED	PROBABLE PER CENT OF TOTAL CO ABSORBED
53	Fatima	Inhaled	S			0.49	1.42	74
54	Fatima	Puffed	S		1.91			
55	Fatima	Inhaled	S	8.1		0.29	0.27	48
56	Fatima	Puffed	S	14.8	0.56			
57	Fatima	Inhaled	B	7.2		0.31	0.48	61
58	Fatima	Puffed	B	20.4	0.79			

absorption is given on a percentage basis showing that from 48 to 74 per cent of the CO of the smoke is absorbed on inhaling the smoke.

DISCUSSION

The experiments with cigarettes given in table 2 show an average yield of 8.3 cc. CO per gram of tobacco. This result is much lower than the results of the investigation shown in table 1 with the exception of Thoms whose use of hemoglobin as an absorbent is entirely inadequate. The I_2O_5 method used is credited with being more accurate than any other, therefore the results of these experiments are dependable. However, a great variation in results must occur due to the different degrees of suction used by different smokers in puffing, etc.

The question of greatest interest is the possible hygienic significance of the retention of CO on inhalation. This retention is undoubtedly due to combination of CO with the hemoglobin of the blood in the lungs. Henderson (10) has recently discussed the affinity of CO for hemoglobin and the rate at which saturation of the hemoglobin takes place. It has long been known that the affinity of hemoglobin for CO is 225 times as great as its affinity for O₂. Haldane (11) has shown that 56 per cent of the first CO drawn into the lungs is absorbed, this corresponding closely with our findings of an average retention of 61 per cent of the CO of smoke in inhaling. The effect of CO on the human being is not a toxic one but is merely a displacement of O₂ from the blood which in extreme cases leads to asphyxiation. The degree of displacement of O₂ from the blood depends on the relative concentrations of CO and O₂ in the air breathed. Haggard and Henderson (12) give the following rough formula for determining the physiological effects of exposure to CO.

(Time of exposure in hours × concentration of CO in parts per 10,000 of air) = 3 or less, no appreciable physiological effect.

= 6, sometimes slight malaise

= 9, headache and some nausea in most people

= 15, dangerous for anything beyond brief exposure

= 15+, dangerous even for brief exposure.

In the last column of table 2 the parts of CO per 10,000 of air are given for tobacco smoke. The number of parts of CO (7.2 to 25.0) is well within the danger zone provided a person were forced to breath the undiluted smoke for over an hour. However this is never the case, the CO is inhaled only at long intervals and many breaths of fresh air are taken in between. At each breath of fresh air the CO tension in the lungs becomes very low and the O₂ tension high, resulting in the displacement by CO from CO-hemoglobin by O₂ to form oxy-hemoglobin. The average amount of CO absorbed per inhalation in our experiments was 0.7 cc., assuming fifteen to twenty breaths per minute as normal and five inhalations of three seconds duration, we have fresh air in the lungs for 75 per cent of the time. Hen-

derson and co-workers (13) have shown that 50 per cent of the CO absorbed is eliminated in an hour upon breathing fresh air. Therefore with fresh air in the lungs for 75 per cent of the time, 37 per cent of the CO would be eliminated. Then assuming that a person smoked continuously for one hour, inhaling five times per minute, i.e., 300 times per hour, the degree of saturation of the hemoglobin would be as follows:

	$300 \times 0.7 = 210$ cc. CO/hour absorbed
37 per cent of 126 cc.	$= 78$ cc. CO/hour eliminated
	132 cc. CO/hour retained
600 cc.	$=$ the normal O_2 capacity of the hemoglobin of an adult (13).

132 cc. CO = 22 per cent saturation of the blood with CO. Therefore in the extreme example given the blood would become 22 per cent saturated with CO. This is an exaggerated example and it is unlikely that smokers habitually inhale as often or continuously as assumed in this case. It is of interest in this connection that all the subjects who inhaled in these experiments experienced unpleasant symptoms afterwards although supposedly immune from long practice to effects of inhaling. This would indicate that smokers who inhale do so only at long intervals or between puffs. Henderson (13) has found that 12 per cent saturation of the blood with CO can be withstood for hours without very disagreeable symptoms, and 18 per cent sometimes results in a headache after an hour's duration. Henderson found that a CO concentration of 2 parts in 10,000 of air finally resulted in a saturation of 12 per cent of the blood. Wahl (8) exposed rabbits for four hours to an atmosphere of tobacco smoke with 2 parts CO: 10,000 of air without ill effect. Since persons seldom inhale as often or as continuously as we have assumed in this discussion, it is extremely unlikely that a degree of saturation of the hemoglobin greater than 12 per cent ever is the result of smoking and therefore this factor (CO) may not be considered as permanently injurious to the smoker.

If the person were a very heavy smoker and in the habit of constantly inhaling, a saturation of the blood close to 10 per cent might be maintained during waking hours. This would reduce

the O₂ capacity of the blood a corresponding amount. It is known (10) that two-thirds of the O₂ of the blood is used when a person is in action and one-third at rest. Therefore a reduction to 90 per cent of the O₂ capacity during activity would be close to the danger level. What effect prolonged 10 per cent saturation might have upon the person is not known.

It is important to know how much CO would be present in the air of a room in which a number of persons were smoking. Assuming that ten persons were smoking continuously producing a maximum amount of CO in a minimum space of 27 cubic meters (a room 3 by 3 by 3 meters), if there were no diffusion, the amount of CO that might be produced in one hour is: Maximum CO per puff (1.91) \times number puffs per hour (300) \times number smokers (10) = 574 cc. or 2.1 parts CO per 10,000 parts of air. This should have no appreciable physiological effect on a non-smoker present in the room.

It should also be pointed out, however, that the affinity of hemoglobin for CO and the rate at which elimination of CO takes place may vary in different individuals (13) also that the volume of blood in children is less than adults and therefore the same amount of CO absorbed would affect a child more seriously than an adult.

CONCLUSIONS

1. The oxidation of I₂O₅ is a favorable method of determining the CO content of tobacco smoke.
2. The amount of CO produced in smoking is increased by strong suction.
3. Cigarettes yield an average of 8.3 cc. CO per gram tobacco smoked, i.e., 0.97 per cent CO by weight.
4. In inhaling tobacco smoke about 61 per cent of the CO is absorbed.
5. Theoretically a *maximum* saturation with CO of 22 per cent of the hemoglobin of a smoker may occur. This degree of saturation cannot be withstood over an hour without disagreeable symptoms (13).
6. It is extremely unlikely that the CO of tobacco smoke is injurious to any but the most inveterate inhalers.

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THE NICOTINE CONTENT OF TOBACCO SMOKE

J. PERCY BAUMBERGER¹

Contribution from the Laboratory of Physiology, Stanford University

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This paper is the second of a series on tobacco smoke in connection with two papers on the effect of smoking on industrial fatigue, which have already been published (1), (2).

INTRODUCTION

It has been the belief of many chemists (Blythe, 3) that nicotine is decomposed in the burning of tobacco in the act of smoking. These chemists believed that the nicotine is decomposed by heat into pyridine, etc., which appear in the smoke and that analytical methods did not distinguish between nicotine and its decomposition products. Van Leeuwen (4) substituted the physiological method for the chemical by studying the effect of injections of tobacco smoke solutions on dogs. Van Leeuwen came to the conclusion that the resultant rise in blood pressure was due to nicotine and not pyridine in the smoke. The physiological method of qualitative determination of drugs is sometimes faulty, therefore it was the purpose of this investigation to reconsider the question of whether or not nicotine is present in tobacco smoke. Rasmussen (5) has reviewed the important methods of analysis of nicotine and shows that the methods of Kissling (6) and Keller (7) are not accurate in that ammonia may be titrated as nicotine and thus introduce a serious error. Rasmussen himself finds the silicotungstic acid method of Chapin (8) more accurate. It is pointed out, however, that in this

¹ The author wishes to acknowledge his indebtedness to Anna C. Franklin for able assistance in this work and to his colleagues Drs. E. C. Franklin and E. G. Martin for advice.

method pyridine will introduce an error unless the method of Thoms (9) of distilling off the pyridine from an acetic acid solution before the nicotine is precipitated, is employed. Thoms and Pontag (10) used the precaution of separating pyridine from nicotine in their studies of tobacco smoke but used the Keller method of determining nicotine which is open to criticism as mentioned above. So far as we can find no investigation of tobacco smoke has used the silicotungstic acid method with the Thoms method of distillation of pyridine. Furthermore, in obtaining the smoke in the experiments of Thoms and Pontag, steady suction was employed, which is a condition not met with in smoking and may effect the nicotine content of the smoke.

METHOD

Our method of determining the nicotine content of smoke is adapted from Rasmussen's method of analysis of tobacco extracts. Two cigarettes were first weighed on a chemical balance and then inserted one in each opening of a Y tube (*a*, fig. 1), the other end of the Y tube being connected directly with a glass tube (*b*) 12 mm. in diameter and 20 cm. long, packed tightly with absorbent cotton. This glass tube is connected with two absorption bottles (*c* and *d*) each containing 10 cc. KOH and 50 cc. ether. The outlet of absorption bottle (*d*) was connected with a water suction pump drawing in the direction of the arrow (*e*). A stop cock (*f*) between *a* and *e* enabled the operator to make the suction intermittent. With stop cock (*f*) closed the suction was started, the cigarettes lighted and then puffed at a normal rate by turning *f* on and off. This process was continued until the cigarettes were smoked down to "butts" of the usual size, which were later weighed.

The process of analysis was as follows: The glass tubing of the apparatus from *a* to *f* was washed out with a mixture of 10 cc. KOH + 50 cc. Ether. This solution was then introduced into a separatory funnel together with the contents of the two wash bottles (*c* and *d*) and the cotton from tube *b* was introduced. After shaking for two minutes the cotton was

withdrawn from the separatory funnel and dried in a Gooch crucible by suction and by pressing with a glass rod on the cotton. The fluid withdrawn from the cotton was added to the contents of the separatory funnel. This whole process took only a few minutes and the distillation was made *immediately* in all cases. The whole solution was analyzed as follows: To one-half, A, of the solution was added an excess of $C_2H_4O_2$ and to the other, B, 20 cc. KOH. The two were distilled by live steam, together with gentle heat, so that the solution in the distillation flasks was reduced to one-third of its volume by the time about 175 cc. of distillate had been collected.

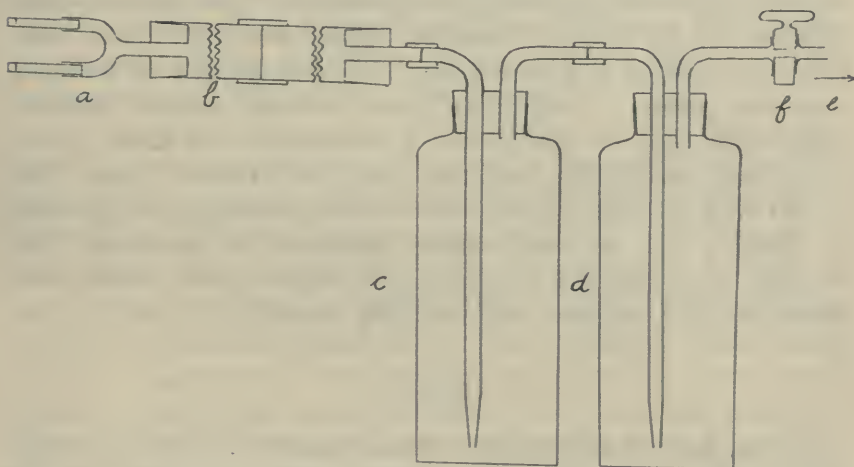


FIG. 1. NICOTINE ABSORPTION APPARATUS

of distillate had been collected. In the case of A all pyridine came over in the first 100 cc. of distillate and traces of nicotine would begin to appear after 175 cc. This process could be followed by testing a few drops of the distillate for nicotine by acidifying with HCl and adding silicotungstic acid. When a precipitate of nicotine silicotungstate appeared the distillation was stopped.

Nicotine distilled over from solution B(KOH), together with pyridine, if present, and the distillation was continued until no opalescence appeared on adding HCl and silicotungstic acid to a few drops of the distillate.

The distillates of A and B were treated separately in identical manner as follows: The distillates were cooled to 30°C., made up to 200 cc. with distilled H₂O, acidified with HCl and 5 cc. of silicotungstic acid were added and the solutions were then placed in shaker bottles and shaken for one hour. The contents of the shaker bottles were immediately passed through Gooch crucibles by suction. The Gooch crucibles had a layer of glass wool on the surface of the floor of the crucible and over this asbestos wool was packed by suction. The glass wool and asbestos wool were prepared by soaking in weak HCl for a day, which was found to decrease their weight appreciably. The crucibles were dried at 70° for forty-five minutes (which was found to be sufficient) and cooled in a desiccator. The first portion of the solution was passed through the Gooch crucible several times to make sure that no precipitate was escaping the filter. After the whole precipitate had been collected 100 cc. weak HCl (1 cc. HCl to 1000 cc. H₂O) were drawn through each crucible to dissolve out any acid soluble precipitate of pyridine. The crucible was then dried at 70°C. for eight hours, cooled in a desiccator and weighed in a weighing bottle.

RESULTS

It was not practicable to use the method of Thoms, in which the pyridine is first distilled off with acetic acid and then the nicotine is precipitated directly, for in such a case the innumerable tarry and resinous compounds in the smoke extract would interfere with the separation of the precipitate. Therefore, the fact that pyridine is not precipitated in very dilute solutions by silicotungstic acid, and furthermore, that the formation of any precipitate of pyridine requires many hours, were used in devising a procedure. It was our purpose to see to what extent nicotine occurs in smoke when the analyses are corrected for pyridine interference. In these experiments the precaution of diluting the distillate to 200 cc. and of allowing only one-half hour for precipitation by silicotungstates to occur, eliminated the pyridine error entirely. This is proven by the fact that no

precipitate of silicotungstates occurred in distillate A where nicotine was absent when treated as above. Distillate A did give a precipitate if undiluted and allowed twenty-four hours for the precipitation to take place. Since no precipitation of pyridine silicotungstate occurred in distillate A, when the above precaution was taken, the precipitate in B would be nicotine silicotungstate, from which the per cent of nicotine in the smoke was calculated. The weight of this precipitate of nicotine-silicotungstate $\times 0.1012$ = weight nicotine. The results are given in table 1 and show that no precipitation of pyridine by silicotungstic acid occurred when the precautions of sufficient dilution and short time precipitation were taken. Chapin (8) discusses the effect of dilution, and Rasmussen (5) the effect of time allowed for precipitation of pyridine silicotungstate.

The per cent of the weight of the tobacco smoke that appears as nicotine in the smoke puffed is given in the next to last column of table 1. The results leave no doubt that nicotine does appear in tobacco smoke in appreciable quantities. The per cent of nicotine for the nine brands of cigarettes studied, is found to vary between 0.358 and 0.998 and the average is 0.573 per cent. As the two brands of cigars smoked were mild and as the suction used in smoking the cigars was not adequate, no importance is given to the cigars smoke analyses. The results obtained in some previous investigations are tabulated in table 2 and show that the average nicotine content of smoke was a little higher than our findings. This discrepancy may be due to the defects referred to above in the methods used or to the use of stronger brands of tobacco. The average for all the results would be about 0.8 per cent of the weight of the tobacco smoked appearing as nicotine in the smoke. The average per cent of the total nicotine appearing in the smoke puffed is about 40, the remainder being accounted for by an increased per cent in the "butt" and by distillation of the burning point which is partly destructive (Habermann). In table 3, the nicotine content of four brands of cigarettes determined by Thurston (17) is given, together with the nicotine content of the smoke of the same brands which was determined by us. Assuming that the nico-

TABLE 1
Nicotine content of tobacco smoke

EXPERIMENT NUMBER	NUMBER SMOKE	BRAND	FORM OF TOBACCO	WEIGHT TOBACCO SMOKE	TIME SMOKE	NUMBER PUFFS	WEIGHT NICOTINE SILICO-TUNGSTATE PP. *	WEIGHT PP. OF PYRIDINE	WEIGHT OF TOBACCO WEIGHT AS NICOTINE	WEIGHT NICOTINE PER PUFF
1†	1	Glad	Cigar	4.636	53	130	0.076	0.0077	0.167	0.000059
2†	1	Presidente	Cigar	9.217	45	241	0.087	0	0.0088	0.000037
4	2	Lucky Strike	Cigarette	0.842		100	0.083	0	0.0084	0.000084
5	5	Lucky Strike	Cigarette	2.419	35	222	0.173	0	0.0175	0.000078
22†	1	Presidente	Cigar	5.380	20	192	0.228	0	0.0231	0.000120
23	6	Lucky Strike	Cigarette	5.006	40	216	0.381	0	0.0386	0.000178
25	6	Egyptian Deities	Cigarette	5.423	20	131	0.204	0	0.207	0.000158
26	6	Bull Durham	Cigarette	2.658	20	192	0.168	0	0.0170	0.000088
28	6	Fatimas	Cigarette	4.983	25	176	0.191	0	0.0196	0.000111
29	6	Home Run	Cigarette	3.331	18	113	0.190	0	0.0192	0.000170
30	6	Palo Alto	Cigarette	3.794	20	116	0.134	0	0.136	0.000117
31	6	Melachino	Cigarette	3.348	20	82	0.146	0	0.0148	0.000180
32	6	Omar	Cigarette	4.344	20	113	0.202	0	0.0204	0.000180
33	6	Piedmont	Cigarette	4.273	20	140	0.232	0	0.0235	0.000168

* Where extract was divided into two parts for distillation, the weight of nicotine-silico-tungstate was doubled.

† Result subject to doubt as explained in text.

TABLE 2

INVESTIGATOR	FORM TOBACCO	PERCENT NICOTINE IN TOBACCO	METHOD SMOKING	PYR- DINE SEPA- RATED BY DIS- TILLA- TION WITH CaH ₂ O ₂	METHOD ANALYSIS	PERCENT OF NICOTINE OF TOBACCO FOUND IN SMOKE	PER CENT OF TOTAL WEIGHT TOBACCO FOUND AS NICOTINE IN SMOKE
Thoms (9).....	Cigar	1.04	Continuous	+	Keller	77.5	0.81
Pontag (10).....	Cigarette	2.0	Continuous	+	Keller	49.1	0.98
Van Leeuwen (4).....	Cigar	0.7-2.2	Puffed	-	Blood pressure	43.7	0.3-0.96
Garner (13).....	Cigar	2.2	Puffed	-	Chapin	27.8	0.61
Lehmann (11).....	Cigar and cigarette	0.6-4.8	Puffed	+	Keller	33.0	0.2-1.6
	Pipe	2.0-2.8	Puffed	-	KI + I	70-80	1.4-2.2
Lancet Laboratory (15)	Cigarette	1.3-1.7	Puffed	-	KI + I	3.7-37	0.05-0.6
	Cigar	0.6-1.2	Puffed	-	KI + I	31.0-83	0.2-1.0
Habermann (14).....	Cigar	1.9	Puffed	-	Kisslings	16.3	0.3
Kissling (16).....	Cigar	0.2-3.75	Continuous	-	Kisslings	44.8	0.09-1.7
All authors.....	Cigar	0.6-4.8				16.3-83.0	0.09-1.7
All authors.....	Cigarette	0.6-4.8				3.7-49.1	0.05-1.6

tine content of these brands of tobacco is about the same as when Thurston made his analysis, from 14.1 to 32.6 per cent of their nicotine appears in the smoke.

TABLE 3

BRAND	PER CENT NICOTINE CONTENT OF TOBACCO*	PER CENT OF WEIGHT OF TOBACCO FOUND AS NICO- TINE IN SMOKE	PER CENT OF NICOTINE OF TOBACCO FOUND IN SMOKE
Fatima.....	2.79	0.393	14.1
Omar.....	1.98	0.469	23.7
Home Run.....	1.78	0.580	32.6
Piedmont.....	3.34	0.550	16.5

*The figures in this column were determined and published by Thurston (17)

DISCUSSION

The method employed eliminates the possible errors due to ammonia and pyridine, and its homologues, which affect the results of other methods. This question is fully discussed in Rasmussen and need not be repeated here. Our analyses, however, show that nicotine occurs in puffed smoke of cigarettes to the extent on the average, of 0.57 per cent of the weights of the tobacco smoked. Cigarettes weigh about 1 gram apiece, and about 80 per cent of the tobacco is smoked. The amount of nicotine in the puffed smoke would be for one cigarette about 0.0057×0.8 or 0.00456 gram. At this rate, four cigarettes would be about equivalent to one cigar (of the mild sort used). The nicotine content of one puff from a cigarette was also calculated and is given in the last column of table 1. Averaging our results, it seems that 0.025 gram of a cigarette are burned at each puff, yielding 0.000137 gram in one puff of cigarette smoke. The rate of puffing a cigar, cigarette, or pipe would therefore determine the nicotine dosage rate for the smoker. Just how much of the nicotine present is absorbed would also have to be known. Lehmann (11) gives a résumé of some experiments he performed on nicotine absorption from tobacco smoke. The smoke puffed automatically was first analyzed and then similar smoke was drawn into the mouth and blown out again into the

apparatus for analysis. The difference in nicotine content was called nicotine absorption. The results were also checked by analysis of saliva of the smoker. The first method gave for nicotine of cigar smoke 34 to 50 per cent absorption, and for cigarette smoke 20 to 37 per cent; the second method gave respectively 70 to 100 per cent and 35 to 42 per cent absorption. Through the electrical precipitation of tobacco smoke, described in another paper of this series, I have been able to show that 66.7 per cent of the smoke and presumably the nicotine drawn into the mouth (puffed) is absorbed, while if the smoke is inhaled 88.2 per cent is absorbed. Assuming an absorption of 88.2 per cent of the nicotine from the smoke inhaled (which is high, as expectoration must rid the body of a great deal of the nicotine) and a rate of inhalation of 5 per minute, the dosage rate of nicotine from a cigarette smoke would be at most $(5 \times 0.000137 \times 0.88)$ 0.000603 gram nicotine per minute, or 36 mgm. per hour. In puffing at the rate of five times per minute with an absorption of 67 per cent the maximum absorption would be $(5 \times 0.000137 \times 0.67)$ 0.000459 gram per minute, or 27.5 mgm. per hour. This maximum absorption is probably seldom reached, as the secretion of saliva is greatly increased during smoking and the consequent expectoration probably eliminates from the body a large part of the 67 per cent which had remained in the mouth, before it has been absorbed. Some smokers, however, do not expectorate during smoking. This matter will be discussed more fully in the paper on electrical precipitation of smoke. Lehmann found that mature habitual smokers could not drink during one-half hour water containing more than 5 to 10 mgm. nicotine without severe symptoms. Non-smokers were affected by 5 mgm. nicotine taken at the same rate. Blythe (3) quotes some experiments of Dvozak and Heinrich in which the author took 1 mgm. of nicotine without severe symptoms, but with 3 to 4 mgm. in forty minutes severe symptoms were obtained, some of which persisted for several days. According to Brundage (12) the fatal dose of nicotine is 500 mgm. taken at one time. It is generally believed that the habitual smoker (18) acquires a partial tolerance for nicotine, but the amount of nicotine drawn

into the mouth in one-half hour, if absorbed at the rate indicated by Lehmann would account for the results in severe illness resulting from smoking by the novice. It would seem very likely that a large portion of the nicotine is dissolved in saliva and expectorated before absorption, as otherwise the tolerance necessary would be extremely high. Dixon and Leek (19) found some evidence of increased destruction of nicotine in the liver of repeatedly injected rabbits which would indicate an acquired nicotine tolerance. The extent to which nicotine poisoning might enter as an effect of habitual smoking would probably depend largely upon the degree of tolerance the subject could develop, the excess to which the habit is carried, and the degree to which inhalation and expectoration are practiced.

In the first paper of this series it is calculated that the CO content of the air in a room 3 by 3 by 3 meters in which ten men smoked for one hour with maximum CO production becomes 2.1 parts CO: 10,000 parts air. This CO content is insufficient to account for the symptoms that non-smokers sometimes experience in such a situation. The maximum nicotine content of the air breathed by a non-smoker in such a room can be calculated as follows:

Number puffs per hour (300) \times maximum nicotine per puff (0.000137) \times number men (10) \times per cent nicotine unabsorbed (0.32) = 0.132 gram. This is equal to 5×10^{-9} grams nicotine per cubic centimeter of air. Assuming a tidal air of 500 cc. and 1200 breaths per hour, 3 mgm. nicotine per hour would be breathed of which 88 per cent or 2.6 mgm. would be absorbed. In this example, however, it has been assumed that the nicotine all remains suspended in the air. This is certainly not the case as the nicotine is in the form of droplets which soon settle out. The dosage that a non-smoker might obtain would therefore be much less than calculated above. However, it is possible that the nicotine absorbed is sufficient to account at least in large part for the headache that many non-smokers have as a result of attendance at a "smoker." It is probable that these headaches are due to increased intracranial blood pressure resulting from the combined effects of nicotine and decreased oxygen supply both of which would tend to raise the blood pressure.

The total smoke absorbed may also be calculated on the basis of the data presented in the paper (third) on electrical precipitation of tobacco smoke. Thus to take the same extremely crowded room and the maximum smoke production: Number puffs per hour (300) \times maximum weight smoke (0.0059) \times number men (10) \times per cent smoke unabsorbed (0.32) = 5.664. This is equal to 21×10^{-8} grams smoke per cubic centimeter air. Assuming a tidal air of 500 and 1200 breaths per hour, 126 mgm. smoke per hour would be breathed of which 88 per cent or 111 mgm. would be absorbed. What physiological effect the smoke may have is not known.

CONCLUSIONS

1. The nicotine content of tobacco smoke can be determined by Chapin's method, with the modifications of Rasmussen without pyridine interference.

2. The average nicotine content of the smoke in per cent weight of tobacco is 0.573 per cent for cigarettes.

3. Of the nicotine in the tobacco about 14 to 33 per cent appears in the smoke puffed.

4. The maximum amount of nicotine retained in the body is calculated as 36 mgm. per hour in inhaling and 27.5 mgm. per hour in puffing. This dose of nicotine would require a high tolerance on the part of the subject in order to escape disagreeable symptoms, and would account for the illness of the novice as a result of smoking. It is undoubtedly true that in most cases, a large per cent of this nicotine is expectorated before being absorbed, therefore the usual dose of nicotine may be very much less than the amount calculated above.

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THE AMOUNT OF SMOKE PRODUCED FROM TOBACCO AND ITS ABSORPTION IN SMOKING AS DETER- MINED BY ELECTRICAL PRECIPITATION

J. PERCY BAUMBERGER¹

Contribution from the Laboratory of Physiology, Stanford University

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This paper is the third of a series on tobacco smoke in connection with two papers on the effect of smoking on industrial fatigue, which have already been published (1), (2).

INTRODUCTION

The investigation was undertaken for the purpose of determining the total amount of smoke produced in smoking tobacco and the per cent of the smoke retained in the body of a subject. The method of smoking by the subject has to be taken into account, and for that purpose it is considered that a person may smoke simply by drawing the smoke into the mouth and then blowing it out again, or by inhaling the smoke into the lungs and exhaling again. There are many other methods of smoking, such as swallowing part of the smoke, or drawing it through the mouth and blowing it out through the nose, but in these experiments only the first two methods are considered and are spoken of as "puffing" and "inhaling," respectively.

In another paper of this series the results of some experiments on the absorption of CO are given and Lehmann (3) has studied

¹ The author wishes to acknowledge his very great indebtedness to his colleague Prof. H. J. Ryan, for the use of the Stanford High Tension Laboratory and for arranging the apparatus necessary to supply a current of high potential. Professor Ryan has also given me his constant expert advice and assistance on electrical questions and has taken a stimulating interest in the experiments. I am also indebted to Dr. E. G. Martin for advice and encouragement. Appreciation should also be expressed for the services of the six men who acted as subjects.

the absorption of nicotine from tobacco smoke. However, no one has, as far as we know, studied the absorption of total smoke.

METHODS

The smoke was collected by the electrical precipitation method of Cottrell (4). The apparatus consisted of a modified Cottrell precipitator similar to the one described by Tolman and co-workers (5). The precipitator is shown in figure 1. The apparatus was made up of a glass tube (*A*) inserted into a hole bored through a section of hard wood (*B*). The wooden base had a hole at right angles to the large glass tube into which a small glass tube (*C*) was introduced. The large tube was held vertical, by burette clamps and a stand support. A piece of aluminum foil (*D*) 0.002 inch thick and 12 cm. long was rolled into a cylinder so that it fitted snugly into the large glass tube (*A*). The aluminum foil made contact with a narrow strip of foil leading to the terminal (*E*). After inserting the foil (*D*), a glass stirrup (*F*), with a piece of tungsten² wire (0.0055 inches in diameter) (*G*) attached, was dropped through the center of the tube. The tungsten wire had a large needle (*H*) attached to one end which served as counter weight to pull the wire through a narrow slit-like opening made by separating two heavy ebonite pieces (*I*) screwed to the lower end of the wooden block. When closed, the two ebonite pieces fitted snugly, a small notch in the exact center, allowing the tungsten wire to pass through. The wire was anchored to an adjustable clamp (*J*) with a binding post (*K*) attached. The aluminum foil served as the electrode of low potential and was grounded through *E*, the tungsten wire was the electrode of high potential received its current through *K*. In determining smoke unabsorbed by puffing or inhaling, the smoke was blown in by mouth through the upper opening of *A* and drawn past the aluminum foil by continuous suction through side tube *C*. For total smoke determination

² Platinum wire was at first used but broke so readily that tungsten wire was substituted, at the suggestion of Professor Ryan, and proved much more serviceable.

the cigarettes were inserted into a rubber stopper fastened on the top of the glass tube *A*, and the subject puffed from the side tube *C*. The precipitate of smoke was deposited on the aluminum foil which was made as light as possible so that the smoke made a large enough increment to make accurate weighing practicable.

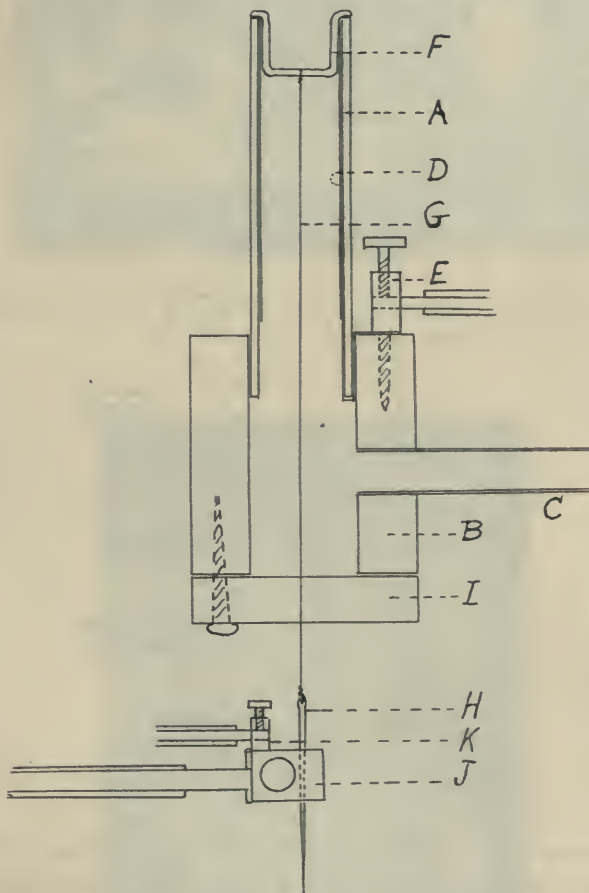


FIG. 1. DIAGRAM OF SMOKE PRECIPITATION

The apparatus was operated from a high voltage direct current source obtained (see figure 2) by stepping up the 110 volt alternating light current supply and rectifying by means of a "Kenotron." Amperage was reduced by inserting in the circuit a 3

mm. tube of tap water one meter long. The direct high voltage current was connected with the central wire (*G*) as cathode of the precipitator and the aluminum foil as anode was connected

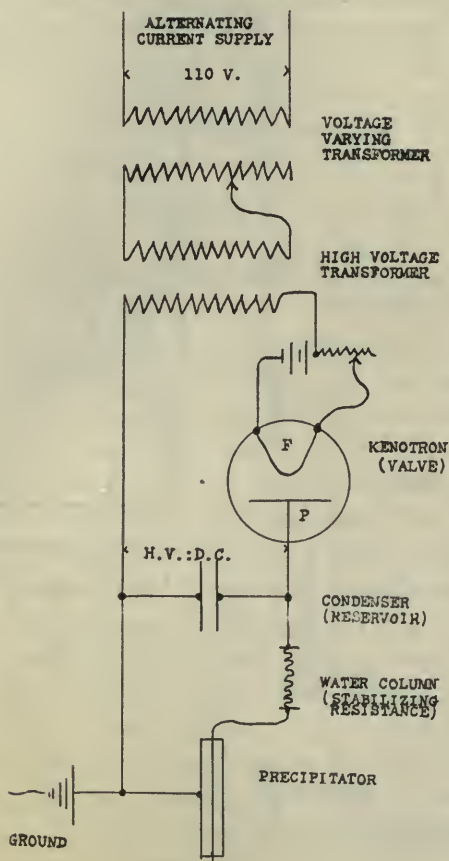


FIG. 2. DIAGRAM OF THE ELECTRICAL CONNECTIONS OF THE SMOKE PRECIPITATOR

The filament and plate of the kenotron are indicated by the letters *F* and *P*, respectively.

to ground. On the "plate" side of the kenotron a 0.004 microfarad mica condenser was connected across the direct current circuit to function as a reservoir from which to deliver an approximately uniform current through the precipitator. The

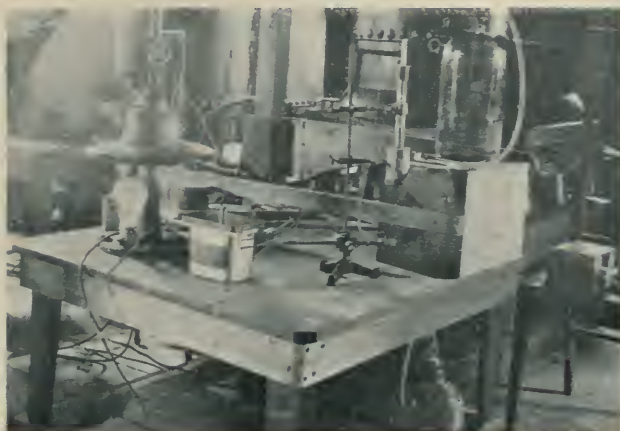


FIG. 3. PHOTOGRAPH OF SMOKE PRECIPITATOR WITH CIGARETTE IN POSITION FOR DETERMINING TOTAL SMOKE



FIG. 4. VIEW OF PRECIPITATOR SHOWING ELECTRICAL CONNECTIONS

apparatus set up is shown in figures 3 and 4. Upon turning on the current a corona discharge took place from the electrode of high potential (wire) to the electrode of low potential (foil). Twenty to thirty thousand volts could be used without sparking over. When sparking took place the experiment was discontinued as otherwise smoke would be lost. The principle involved is, that given a needle-point electrode (in this case the central wire) of high potential and a large flat electrode of low potential, the air between becomes highly charged with electricity of the same sign as the needle point. The particles or droplets suspended in the air will take up the charge and be driven to the large electrode. The greater the potential gradient between the central wire and the aluminum foil, the greater the speed at which the particle travels.

The efficiency of the apparatus can be measured by the degree to which the smoke passes beyond the first point of electrification. This point is situated about 2 cm. from the entrance to the glass tube and 1.5 cm. from the beginning of the foil, due to the stirrup to which the central wire is attached. In the beginning of the foil so that about 8 cm. of foil was left free from precipitate. This showed that the suspended material in the smoke was completely precipitated.

Subjects were used in all the experiments and an attempt was made to have the same subject in the puffing as well as the inhaling experiments, with one brand of cigarette. The number of puffs or inhalations was counted by the operator with a tally register. The foils were weighed before hand and a different foil used for each experiment. After the experiment the foils were dried over CaCl_2 for twenty-four hours and weighed. The weight of the foils averaged 750 mgm., and as the usual precipitate was over 10 mgm. accurate weighings could be made. The precipitate was washed off the foils with ether and liquid soap, and the foil was then dried with alcohol, followed by ether and held in the air current of an electric fan. The weight of the foils remained practically unchanged during the course of the series of experiments. The cigarettes used were weighed and numbered before the experiment and the butts were dried and weighed afterwards.

EXPERIMENTS

Experiments 1, 3, 6, 8, 12, 14 and 15 on table 1 give the amount of smoke blown out of the mouth after puffing; experiments

TABLE 1

BRAND	WEIGHT TOBACCO	WEIGHT PRECIPITATE	NUMBER PUFFS	NUMBER INHALATIONS	PER CENT OF WEIGHT OF TOBACCO IN PRECIPITATES	WEIGHT UNABSORBED SMOKE		WEIGHT TOTAL SMOKE PER PUFF	NUMBER MINUTES	SUBJECT	NUMBER EXPERIMENT
						Per puff	Per inhalation				
Melachrino.....	0.769	0.045	20		5.80	0.0022				T.	1
Melachrino.....	0.792	0.014		12	1.80		0.0012			T.	2
Melachrino.....	0.868	0.096	20		11.05			0.0048	6	Ba.	25
Melachrino.....	0.843	0.113	22		13.41			0.0051	4	Ba.	28
Melachrino.....	0.767	0.074	20		9.65			0.0037	5	Ba.	26
Melachrino.....	0.866	0.090	31		10.39			0.0029	6	Ba.	29
Melachrino.....	0.810	0.091	24		11.23			0.0038	5	Ba.	27
Home Run.....	0.600	0.025	12		4.20	0.0021				J.	3
Home Run.....		0.001		1			0.0010			J.	4
Home Run.....	0.565	0.018		16	3.19		0.0010		9	B.	31
Home Run.....	0.572	0.064	16		11.19			0.0040	4	Ba.	23
Home Run.....	0.718	0.093	16		12.95			0.0058	4	Ba.	24
Omar.....	1.678	0.008		51	0.50		0.0001			A.	9
Omar.....	0.700	0.013	×		1.90	×				L.	6
Omar.....	1.070	0.088	34		8.23			0.0026		Ba.	19
Pall Mall.....	0.855	0.004		21	0.50		0.002			H.	16
Pall Mall.....	0.841	0.026	14		3.10	0.0019			7	S.	15
Pall Mall.....	0.816	0.002		19	0.20		0.0001		7	S.	18
Pall Mall.....	1.133	0.160	23		14.12			0.0069	4	Ba.	22
Pall Mall.....	1.062	0.172	35		14.50			0.0049	4	Ba.	21
Egyptian Dieties...	1.102	0.005		22	0.45		0.0002		13	B.	32
Egyptian Dieties...	1.047	0.056	×		5.35	×				R.	12
Egyptian Dieties...	1.227	0.066	28		4.57			0.0024		Ba.	13
Egyptian Dieties...	1.080	0.018	18		1.70	0.0010			10	T.	14
Lucky Strike.....		0.009	9			0.0010				A.	8
Lucky Strike.....	0.907	0.063	24		6.95			0.0026	8	Ba.	20

2, 4, 9, 16, 18, 31 and 32 give the amount of smoke exhaled after inhaling and experiments 13, 19 and 20 to 29 give the total amount of smoke drawn from a cigarette by puffing. As a record

of the number of puffs or inhalations was kept it was possible to calculate the approximate amount of smoke per puff or inhalation. The weight of the smoke precipitated is given in column 3 of table 1, and this weight is converted into per cent of the weight of tobacco smoked and given in column 6. The percentage of smoke varies from 0.2 to 14.5 per cent and the weight per puff from 0.0001 to 0.0069 gram. These great differences are due to the manner of smoking which result in different degrees of absorption of the smoke. This fact is brought out in

TABLE 2

BRAND	AVERAGE PER CENT OF WEIGHT OF TOBACCO AS SMOKE			AVERAGE PER CENT OF TOTAL SMOKE DRAWN INTO MOUTH		AVERAGE GRAMS SMOKE PER PUFF		
	Drawn into mouth	Puffed out of mouth	Ex- haled after in- haling	Ab- sorbed in puffing	Ab- sorbed in in- haling	Drawn into mouth	Ab- sorbed in puffing	Ab- sorbed in in- haling
Melachrino.....	11.15	5.8	1.8	47.9	83.8	0.0041	0.0019	0.0029
Home Run.....	12.07	4.2	3.2	65.2	73.7	0.0049	0.0028	0.0039
Omar.....	8.23	1.9	0.5	76.9	93.9	0.0026		0.0025
Pall Mall.....	14.31	3.1	0.2	78.3	98.6	0.0059	0.0040	0.0058
Egyptian Dieties.....	4.86	1.7	0.4	65.0	90.8	0.0024	0.0014	
Lucky Strikes.....	6.95					0.0026	0.0016	
Grand averages.....	9.59	3.3	0.6	66.7	88.2	0.00375	0.0023	0.0037

table 2 where the experiments are classified. The amount of smoke that would be drawn into mouth averages 9.59 per cent (by weight of the tobacco smoked) while the smoke puffed out of the mouth averages 3.3 per cent and smoke exhaled after inhaling averages 0.6 per cent. Undoubtedly the difference in amount of smoke puffed out or inhaled is equal to the amount of smoke retained in the body. On this assumption, which seems to be beyond question, the amount of smoke absorbed in puffing and in inhaling can be calculated. These calculations are given in columns 5 and 6 of table 2. The smoke absorbed in puffing averages 66.7 per cent (of the total smoke) and in inhaling 88.2 per cent. In a similar manner the weight of smoke probably retained in the body per puff or per inhalation can be calculated

and in the last two columns of the table these averages are given as 0.0023 gram per puff and 0.0037 gram per inhalation.

DISCUSSION

That a retention of smoke as high as 66.7 per cent in puffing and 88.2 per cent in inhaling should occur is natural enough when we consider the processes involved. In puffing, a form of smoking, the buccal cavity is made air tight (Murlin, 6) and a negative pressure is produced by depressing the tongue and the lower jaw. The smoke is thus drawn into a closed compartment with many ridges and irregularities all moist with saliva. This would be an ideal condition for absorption of the smoke which consists of a suspension of liquid particles, for as is well known such a suspension settles out more readily when at rest than when in motion and will readily precipitate on any baffles which may be encountered. In inhaling the suction may be from the mouth as in puffing, followed by an expansion of the lungs and opening of the epiglottis so that the smoke is drawn into the lungs or the suction may be due primarily to the expansion of the lungs acting directly as a suction on the cigarette. In either case the smoke would be drawn through a tortuous passage in which solution of the smoke in the saliva and on mucous membranes could very well take place. In both puffing and inhaling it would seem remarkable that all the smoke is not dissolved. However, smoke is very difficult to absorb and in some experiments tobacco smoke has been drawn through as many as eight wash bottles of a suitable solvent without complete absorption. This fact was largely responsible for the selection of the electrical precipitation method.

The smoke absorbed in puffing and inhaling is a very complex mixture of substances and has been shown to contain CO, HCN, NH₃, nicotine, pyridine and their derivatives, tar and resinous compounds, etc. In the smoke as precipitated probably only the suspended solid and liquid particles are involved. This would include nicotine as it is in a suspended state and tests with silicotungstic acid gave a very heavy precipitate of nicotine silicotungstate. Analysis of the precipitate deposited on the

aluminum electrode would be a very suitable way of determining nicotine in tobacco smoke and such analyses should be made. In a paper, second of this series, on the nicotine content of tobacco smoke the absorption of nicotine has been considered on the basis of the absorption of smoke determined in this paper by the electrical method for it is undoubtedly true that nicotine and total smoke would be retained in the same proportion. With this assumption as pointed out in the paper on nicotine, 66.7 per cent of the nicotine would be absorbed in puffing and 88.2 per cent in inhaling. As we found an average of 0.57 per cent nicotine in the smoke of cigarettes, it was calculated that as much as 0.036 gram of nicotine per hour of cigarette smoking could be absorbed in inhaling and 0.027 gram per hour in puffing. This amount of nicotine is far in excess of the amount Lehmann (3) found could be drunk by habitual smokers in an equal period of time, therefore it seems that the amount of nicotine actually absorbed by the body (and not merely temporarily retained) may be less than calculated from our figures. Solution of the smoke in saliva and secretions of the mucous membranes of the throat must undoubtedly take place to a very large extent as pointed out above. This would be also aided by the excessive secretion of saliva under the influence of nicotine. The habit of expectorating would rid the body of a large part of the retained nicotine before total absorption could take place through the mucous linings. However, many smokers do not expectorate while smoking. Some absorption undoubtedly does take place as nicotine has been detected in urine, saliva and perspiration, and furthermore there is much clinical evidence (Cushing, 7) that indicates nicotine absorption.

CONCLUSIONS

1. The electrical precipitation method can be used to determine the total amount of suspended solid and liquid particles, including nicotine and pyridine, in tobacco smoke.

2. The smoke drawn into the mouth in smoking has a weight of 9.59 per cent of the tobacco burned. In puffing 66.7 per cent of the smoke is retained in the body of the subject and in inhaling 88.2 per cent is retained.

3. Probably a large per cent of the smoke retained in the body is removed by expectoration before absorption of nicotine has been completed.

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CHRONIC INTOXICATION BY SMALL QUANTITIES OF CADMIUM CHLORIDE IN THE DIET¹

CARL O. JOHNS, A. J. FINKS AND CARL L. ALSBERG

*From the Protein Investigation Laboratory, Bureau of Chemistry, United States
Department of Agriculture, Washington*

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As explained in the paper by Schwartz and Alsberg (1) printed elsewhere in this Journal, at one period of the war it was proposed to substitute cadmium for tin in solder for food containers. The study herein reported, as well as that by Schwartz and Alsberg, was undertaken to determine the toxic effects of small amounts of cadmium ingested with food. Such information was lacking, as examination of the literature showed. For such an analysis of the literature the reader is referred to the paper by Schwartz and Alsberg.

The only information available relates to the toxicity of considerable dosages of cadmium. Since the quantities of cadmium that would be likely to contaminate food packed in tin containers would undoubtedly be very small, such information was not significant in judging the possible toxicity of small quantities consumed over long periods. The ordinary pharmacological methods of determining the toxicity of substances therefore were not applicable to the problem under consideration. It was necessary to determine the effect of very small quantities given *per os* over long periods. The simple feeding of an ordinary diet containing minute quantities of the substance to be tested, such as has been practiced by previous investigators, does not always give clean-cut indications of toxic effects, since such feeding is invariably accompanied by variability in the food and the food intake and there are no sharp criteria to determine the effect

¹ Presented before the Society for Pharmacology and Experimental Therapeutics, April 24, 1919.

produced. It therefore occurred to the authors that more decisive results might be obtained by adopting the technique developed by such students of nutrition as Osborne and Mendel and McCollum in determining the food value of given elements in the diet. This method consists, as is well known, in feeding the given diet to growing rats and judging the effect of the diet by the growth curve of growing animals. It was thought that, since this technique has been so thoroughly standardized and the growth curve of white rats so thoroughly determined, this procedure would be preferable to that used by previous investigators for similar purposes. After the value of the method and preliminary results obtained with cadmium were reported (2), the same method was used independently by Sollmann (3) with good results.

EXPERIMENTAL

Preparation of diets

The basal diet was one with which the authors have had considerable experience and which has proved fully adequate for the normal growth of albino rats. It had the following composition:

	grams
Cooked soy bean meal.....	50
Salt mixture ²	4
Starch.....	21
Lard.....	18
Butter-fat.....	7

The cadmium was incorporated in the diet by adding the required quantity of a standard cadmium chloride solution. This standard solution contained the equivalent of 0.05 gram metallic cadmium per cubic centimeter. A suspension of the salt mixture was made with water to which was added the requisite amount of cadmium. The salt mixture was then slowly evaporated to dryness and ground to a very fine powder which was thoroughly mixed with the soy bean meal and starch. The lard and butter-fat were melted and added to complete the diet.

² For the composition of the salt mixture, see Osborne, T. B., and Mendel L. B.: Jour. Biol. Chem., 1917, xxxii, 374.

Discussion of results

Various concentrations of cadmium, calculated as the metal and ranging from 62.5 to 1000 parts per million, were incorporated in the basal diet, and the diet tested. The rats receiving 1000 parts per million in the diet did not grow and died in about thirty days; those receiving 500 parts per million also failed to grow, but lived about one hundred and forty days; while those receiving 250 parts per million grew slightly, then decreased in

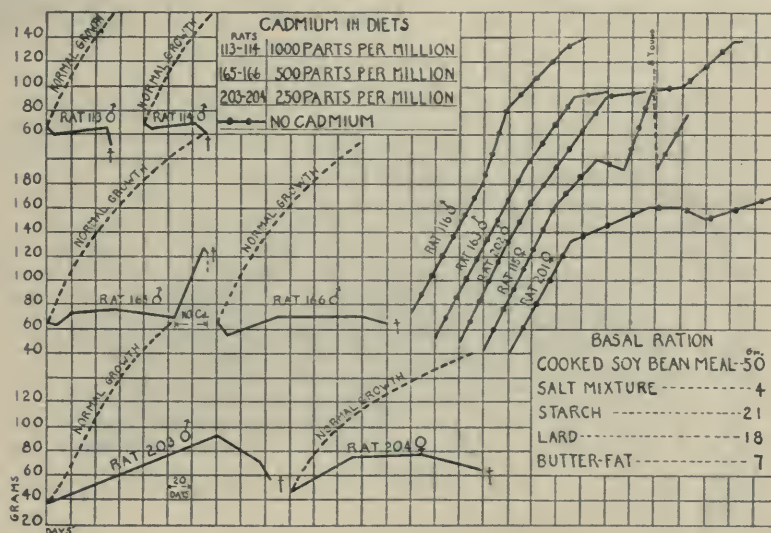


CHART 1

weight, and finally died at the end of about one hundred and eighty days. Rat 165 ♂ was placed on the basal diet, free from cadmium, at the end of one hundred and five days. It then grew rapidly for about twenty days, when it suddenly declined and died. It had perhaps become permanently injured during the time that it received cadmium. These results are shown graphically on chart 1.

When the diet contained 125 parts per million of cadmium the rate of growth for some time was almost normal. Most of the male rats died in about fifty days, although they grew fairly well

until just before their death. It is interesting to note that three females survived a long time, one of them dying at the end of one hundred and ninety days, while another was still alive at the end of two hundred and eighty days when the experiment was discontinued. The results of these experiments are recorded on chart 2.

Decreasing the quantity of cadmium to 62.5 parts per million resulted in the normal rate of growth and no deaths ensued. The average daily intake of food in this case was 9 grams and that of the cadmium was 0.56 mgm. The growth curves are shown on chart 3.

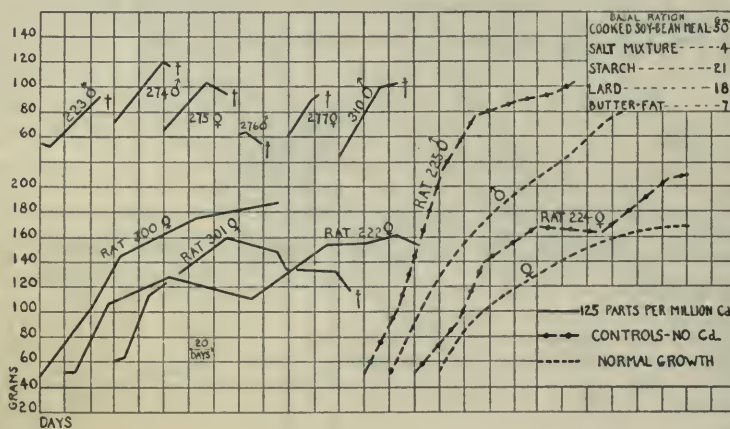


CHART 2

The quantity of cadmium present in the diet had a marked influence on the amount of food eaten. When the diet contained 1000 parts of cadmium the average daily food intake was 3 grams and the intake of cadmium was 3 mgm. Decreasing the quantity of cadmium in the diet to 500 parts per million resulted in a daily average intake of 4.5 grams of food and 2.2 mgm. of cadmium. A diet containing 250 parts of cadmium per million was ingested to the extent of 4 grams per day, which contained 1 mgm. of cadmium. When the cadmium content of the food was 125 parts per million, the food intake was 6.4 grams per day and the cadmium intake was 0.8 mgm. The lowest concentration of

cadmium tested, 62.5 parts per million, had no effect on the growth of the rats. They ate an average of 9 grams of food per day, which contained 0.56 mgm. of cadmium. The average daily food intake on the basal diet was 9.5 grams. It will be noted from the above data that the food intake increases as the quantity of cadmium in the diet is decreased.

Unquestionably, therefore, a large part of the effects of feeding cadmium chloride under the conditions obtaining in this investigation is to be attributed to the fact that cadmium chloride in the

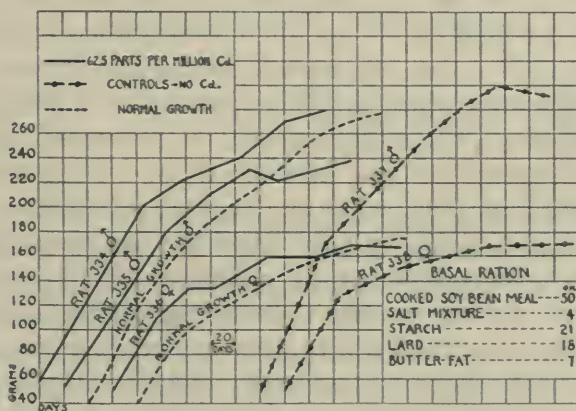


CHART 3

three higher concentrations used reduced the food intake. That such concentrations should have this effect is quite in harmony with the observations of Schwartz and Alsberg on cats, for the concentrations affecting the growth of the rats are of the same general order of magnitude as the concentrations which they found caused vomiting by cats. The vomiting by cats generally observed by these authors was not noticed in our experiments with rats. The observations made in the present investigations furnish no conclusive evidence to determine whether or not cadmium had any effect other than the reduction of the food intake below the minimum needed for growth and maintenance. It is clear however, that doses of cadmium as small as 0.56 mgm. per day produce no effect upon the growth of rats and are without

apparent cumulative action. This is quite in harmony with the observations of Schwartz and Alsberg who were unable to observe any cumulative effect upon cats.

SUMMARY

Various concentrations of cadmium chloride were incorporated in a diet which was known to be adequate for the normal growth of rats. Diets containing from 1000 to 62.5 parts per million of cadmium were tested. Very little or no growth occurred and death ensued when the concentration of cadmium was 1000, 500, or 250 parts per million. When there were 125 parts per million of cadmium in the diet, the initial rate of growth was normal. All of the male rats receiving this concentration of cadmium died in about fifty days, while the majority of the females survived a very much longer time, one of them dying at the end of one hundred and ninety days, while another was still living at the end of two hundred and eighty days when the experiment was discontinued. A concentration of cadmium of 62.5 parts per million had no effect on growth, the rate of growth and food intake being normal. The food intake increased as the concentration of cadmium in the diet was decreased. Whether the interference with growth was due solely to the diminished food intake was not determined. With a dosage of 0.56 mgm. of cadmium per day no cumulative action was observed.

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THE ACTION OF FURFURAL¹

HUGH McGUIGAN

*From the Laboratory of Pharmacology and Therapeutics, University of Illinois,
College of Medicine, Chicago*

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Relatively few investigations of the action of furfural (of
furfurol), $\begin{array}{c} \text{HC} \text{---} \text{CH} \\ || \quad || \\ \text{HC} \quad \text{C} \cdot \text{CHO} \\ \diagdown \quad / \\ \text{O} \end{array}$, have been made. This is probably

due to the fact that it has only recently been much used in the industries. Its reaction with phenol is now taken advantage of in the manufacture of certain artificial resins in which it takes the place of formaldehyde.

Laborde and Magnan (1), in studying the toxicity of industrial alcohol, made a brief study of the action of furfural which is found as a by-product of the alcohol. They found, in two experiments with dogs, that the intravenous administration of furfural caused epileptic-like spasms which were followed by paralysis. They also found that 4 cc. of furfural when given by mouth was fatal to a dog weighing 6 kilos.

Lepine (2), and also Cohn (3) made incomplete studies of its action on animals. Jaffé and Cohn (4) studied its excretion products on dogs and fowls. They identified pyromucic acid and two other compounds; one they named pyromucuric acid which is a conjugated form of pyromucic acid analogous to hippuric acid. They considered the third compound, which is water soluble, to be a conjugated compound of glycocholic acid and furfuracrylic acid.

¹ The furfural used in this investigation was prepared by The Miner Laboratories, Chicago, and had a boiling point of 160°C. and specific gravity of 1.1598 at 4°C.

Joffroy and Serveaux (5) administered furfural intravenously to dogs, rabbits and guinea pigs in order to determine its toxicity. They make a distinction between true or absolute toxicity and experimental toxicity. They define the former as the actual quantity of a drug that must be injected in order to kill quickly the majority of animals. They define the latter as the quantity per kilo of body weight required to kill an animal when the drug is injected intravenously at a uniform rate until death.

They found that the true toxic dose of furfural for dogs was a little above 0.20 gram per kilogram of body weight; about 0.14 per kilogram for rabbits and about 0.17 per kilogram for guinea pigs. I accept these figures as essentially correct, and agree with their observations. However, from a practical point of view, the estimation of toxicity by this method, is not so valuable as when the drug is given by mouth. Both methods, however, should be studied.

Hans Weiss (6) reports first a stimulation, later paralysis of the respiratory center, motor and reflex paralysis, convulsions, salivation, solution of red blood corpuscles, and lowering of body temperature.

The present investigation is a continuation of these earlier investigations and is concerned with the action of furfural on bacteria, yeast, gold fish, frogs, white mice, rabbits, cats, and dogs.

THE ACTION OF FURFURAL ON BACTERIA²

The Anderson and McClintic (7) modification of the Rideal-Walker method for the determination of the phenol coefficient was used as a measure of the action of furfural on bacteria.

A 24-hour broth culture of *Bacillus typhosus* which had been transplanted daily for five days was used. The culture was thoroughly shaken just before using and filtered through sterile paper. Three determinations were made with the following results. 0.26, 0.27, 0.26,—average, 0.263.

² This part of the investigation was made by Messrs. R. L. Groves and R. C. Robertson of the Department of Bacteriology.

ACTION OF FURFURAL ON YEAST

The change in the rate of fermentation of a dextrose solution by yeast was taken as a measure of the action of furfural on yeast. Five grams of Fleischmann's compressed yeast was suspended in 100 cc. of 0.9 per cent sodium chloride solution and a portion of this mixed with an equal volume of 4 per cent dextrose solution. Fifty cubic centimeters of this mixed solution having a dextrose concentration of 2 per cent and a yeast concentration of 2.5 per cent was incubated, with the quantity of furfural given below, at 40°C. in all cases.

The concentration of the furfural solutions were as follows:

	TUBE				
	1	2	3	4	5
Furfural per cent.	2.0	1.0	0.5	0.2	0.0
Gas formed in two hours, cc.	0.0	1.0	3.0	3.8	3.5

It is thus seen that 0.2 per cent furfural does not inhibit fermentation in two hours that 2 per cent furfural inhibited fermentation completely, and 1 per cent was less harmful than 2 per cent but more harmful than 0.5 per cent.

In twelve hours all except the strongest solution had fermented. We may take it, therefore, that a 1 per cent furfural solution delays, but does not prevent, the action of yeast. A concentration of 2 per cent is necessary to entirely prevent fermentation. More dilute concentrations of furfural do not hasten the rate of fermentation.

THE ACTION OF FURFURAL ON GOLD FISH

The toxic concentration of furfural was determined for gold fish by placing four of them in 1000 cc. of each of the furfural concentrations given on following page.

The gill surface of different fish is approximately proportional to their weight, therefore, it is unnecessary to weigh fish when used as test animals as above.

EXPERIMENT NUMBER	FURFURAL	HOURS UNTIL DEATH
	<i>per cent</i>	
1	0.250	0.25
2	0.125	1.25
3	0.100	1.50
4	0.050	6.00
5	0.025	10.00
6	0.010	12.00
7	0.005	Survived

THE ACTION OF FORMALDEHYDE ON GOLD FISH

The toxicity of formaldehyde for gold fish was next determined in order to give a comparison with furfural. Commercial formalin containing about 39 per cent formaldehyde was diluted and the fish placed in 1000 cc. of the solution having the concentrations given below:

EXPERIMENT NUMBER	FORMALDEHYDE	HOURS UNTIL DEATH
	<i>per cent</i>	
1	0.04	1.5
2	0.02	2.0
3	0.012	3.0
4	0.08	18.0
5	0.004	18.0

These results indicate that furfural is about one-third as toxic to gold fish as formaldehyde.

Some additional experiments performed under similar conditions are as follows:

EXPERIMENT NUMBER	SOLUTION	PER CENT	DEATH IN HOURS	NUMBER OF FISH USED
1	Phenol	0.010	6.5	2
2	Phenol	0.015	3.0	2
3	Phenol	0.030	2.3	1
4	Furfural	0.030	4.5	2
5	Furfural	0.045	3.5	4
6	Furfural	0.100	1.3	1
7	Formaldehyde	0.020	2.5	4
8	Formaldehyde	0.030	1.2	1

These results show that furfural is about one-half as toxic for gold fish as phenol, and about one-third as toxic as formaldehyde for the same animals.

THE ACTION OF CONCENTRATED FURFURAL ON FROGS

I. One-tenth cubic centimeter of furfural was dropped into the mouth of a 34-gram frog. This caused a marked paralysis in three minutes. The action in the main resembled chloral. The animal died in forty minutes and the heart was found stopped in diastole.

II. Into the lymph sac of another animal weighing 22 grams 0.5 cc. furfural was injected. Death resulted in ten minutes.

III. One-tenth cubic centimeter of furfural dropped onto the back of a frog weighing 26 grams caused paralysis in four minutes to such an extent that the animal could not turn over. After an hour the frog was still in the same condition and the heart was beating feebly.

THE ACTION OF DILUTE SOLUTIONS OF FURFURAL ON FROGS

Solutions of furfural were injected into the anterior lymph sac of frogs weighing about 30 grams as follows: IV, 2. cc., 5 per cent; V, 1 cc., 5 per cent; VI, 2 cc. 2.5 per cent; VII, 1 cc., 2.5 per cent; VIII, 1 cc., 1 per cent; IX, 0.5 cc., 1 per cent.

After two minutes nos. IV, V, and VI, were completely paralyzed. The pupils were constricted as after a large dose of chloral. After ten minutes no. VII was paralyzed in the hind legs but held up its head. The pupils were dilated. After fifteen minutes nos. VIII and IX were but slightly affected. They turned over when placed on back, but were clumsy. After twenty-five minutes the eyes of IV, V, VI, and VII were still constricted and the animals completely paralyzed. No. IV died in thirty-five minutes. The auricles were much dilated. In nos. IV and V the ventricles were found in systole; in VI and VII both auricles and ventricles were in diastole. After forty minutes nos. VIII and IX were hyper-irritable with some motor impairment. They completely recovered from this dose.

Frogs weighing 43 grams were injected into the sciatic region with 0.25 and 0.5 cc. of 5 per cent furfural. In five minutes the injected leg was paralyzed in each animal. Sensation was more influenced than motion. There was a total anesthesia of the leg involved. Afterwards these developed a highly irritable condition resembling that caused by picrotoxin. This was still evident after twelve hours. These animals recovered from this dose.

After removal of the cerebrum twitching still persisted. When the medulla was removed spasms ceased practically and did not return showing that the main action is on the medulla.

THE ACTION OF FURFURAL ON WHITE MICE

White mice weighing about 21 grams were used in this series. They were given 1:1000 furfural by hypodermic injection. The first mouse showed a rapid jerky respiration after the injection of 0.25 cc. The animal was hyper-irritable and jumped when touched. The tail for a time was straight and rigid, but not elevated. In a short time the animal became drowsy.

The second mouse injected with 0.5 cc. showed the same symptoms. In both cases there was a tendency to scratch the nose with the fore feet much like animals into whose fourth ventricle atropine or morphine had been injected. The animals recovered from this dose.

The third and fourth mice were injected with 0.25 and 0.5 cc. of 1:250 furfural. This concentration caused more pronounced depression; otherwise, the symptoms were similar to the mouse injected with 1:1000 furfural. These mice completely recovered.

A fifth mouse was injected hypodermically with 0.25 cc. 1 per cent furfural. Defecation resulted and a quick depression with labored jerky respiration which soon became infrequent—about once in ten seconds. In twenty minutes this dose caused death, but double the dose or 0.5 cc. 1 per cent was not more quickly fatal.

ACTION OF FURFURAL ON RABBITS

Lepine (2) and Cohn (3) each administered aqueous solutions of the drug hypodermically. It seemed better to drop it into

the mouth. Owing to the small dose required, one can, by using a finely graduated pipette, administer the drug in this way without loss.

Cohn states that when 0.15 to 0.2 gram are administered subcutaneously to medium sized rabbits, the symptoms are unsteady gait, inability to stand and then paralysis. Respiration was at first frequent and irregular, finally depressed and sometimes pronounced dyspnea and cyanosis resulted. The heart beat was regular and strong. The reflexes were at first exaggerated, then paralyzed. Weak clonic convulsions may supervene from asphyxia.

Lepine's observations are essentially the same. He states that 0.05 gram per kilo is not fatal. A dose of 0.08 gram per kilo elicits grave symptoms and 0.1 gram per kilo always caused death within two days.

In my experiments in which the drug was administered by mouth, 0.25 cc. per kilogram was without noticeable effect, 0.33 cc. per kilogram had no effect except to stimulate respiration, and perhaps cause some drowsiness. Five-tenths cubic centimeter per kilogram was a little more pronounced while 0.7 cc. per kilogram caused immediate paralysis. This is best illustrated in the following protocol:

A dose of 0.8 cc. per kilogram given by mouth was fatal within fifteen hours. A healthy rabbit weighing 1200 grams was given 1 cc. furfural by dropping it into the mouth. Two minutes later it was paralyzed and fell on its side; the pupils were dilated and it had no reflexes of its skeletal muscles; the corneal reflex was positive and respiration was rapid and deep. After eight minutes respiration was increased and irregular. After thirty minutes the condition was apparently improving, and respiration became slower. After seventy-five minutes the condition was such that recovery was expected. The next morning the animal was found dead but still warm.

With larger doses causing death in about ten minutes, the respiration was at first rapid, but soon becomes slow and labored. The heart rate was rapid and the amplitude decreased; the ears were cold. The pupil varied in size; at first it usually dilated; then constricted, but as death approached it again dilated.

A rabbit weighing 1600 grams was given 0.5 cc. furfural per kilogram by mouth. In two minutes there was depression—partial paralysis, increased jerky respiration from which the animal completely recovered. After four days the animal was normal.

A second rabbit weighing 1850 grams was given 0.7 cc. furfural per kilogram. It fell on its side paralyzed. Respiration was stimulated, and reflexes in skeletal muscles were much depressed. After six minutes the pupil was constricted from 10 to 5 mm. and respiration was deep and regular. After ten minutes it recovered partially and stood up; after forty-five minutes it recovered still more. The heart beat was rapid and weak. After seventy-five minutes the pupils dilated. Next day the rabbit was almost normal and in four days complete recovery took place.

When a few drops of a 5 per cent solution are instilled into the conjunctival sac, total anesthesia of the cornea ensues in a few minutes. There is some pain caused by the first instillation, but a second instillation is painless. If one holds a few cubic centimeters of such a solution in the mouth, anesthesia of the mucous membranes and tongue results. These results are confirmative of Cohn's findings.

THE ACTION OF FURFURAL ON CATS

A young cat weighing 700 grams was given 1 cc. of 5 per cent furfural hypodermically, and another young cat of the same weight was given twice this quantity. There was no effect except a slight drowsiness with the larger dose.

A large cat weighing 3.5 kgm. was given 5 cc. of 5 per cent furfural equivalent to 0.25 cc. actual furfural. This animal showed no effects after thirty minutes and it was then given 0.5 cc. of the concentrated preparation with the results showing in the following protocol:

- 2:00. 0.5 cc. furfural injected hypodermically.
- 2:05. Animal very irritable and excitable, apparently due to local pain.
- 2:08. Deep, regular respirations, some salivation, drowsy.

- 2:10. Repeated vomiting.
- 2:12. Paralysis, pupils constricted.
- 2:20. Slight movement but unable to stand on feet.
- 2:23. Spasmodic twitchings of head, feet and tail.
- 2:25. General spasm with head back and feet drawn against abdomen.
There were five spasms beginning at the head end, with intervals of relaxation intervening. These spasms were almost as violent as strychnine tetanus but recovery took place very rapidly. It appears to be complete and eventually leads to sleep.

The difference in action between 0.25 cc. and 0.5 cc. was marked. This difference is not due to the concentration of the furfural as is shown below.

The large cat was given hypodermically 20 cc. 5 per cent solution of furfural. The symptoms were essentially the same as described above. Marked salivation, vomiting, and spasmodic twitching commenced in thirty minutes. These were about the same intensity and duration as described above with a corresponding dose. The two smaller cats had slight spasms, but soon recovered. The main effect was soporific. In one hour they had practically recovered. In one hour and thirty minutes the larger cat was able to sit up. The next day all of the cats were normal.

ACTION OF FURFURAL ON DOGS

Jaffé and Cohn found that dogs oxidized furfural to the corresponding acid (pyromucic). They found that dogs (weights not given) could tolerate 5 to 6 grams per day without showing symptoms for weeks. They do not record the symptoms or the relative dose.

Experiment I. In an anesthetized dog, weighing 7 kgm., I intravenously at intervals, 0.1 cc., 0.5 cc., and 1 cc. in the course of thirty minutes. The smaller doses caused no apparent change. Five-tenths cubic centimeters caused a drop in the blood pressure of 40 mm. Hg. There was little influence on respiration. One cubic centimeter caused a profound drop in blood pressure, and respiration stopped before the heart beat. The heart was later

found to be dilated. The stoppage of respiration before the heart was corroborated in other experiments (confirmatory of Joffroy and R. Serveaux, also Laborde and Magnan).

Experiment II. Dog weighing 9 kgm. 5 cc. of furfural in 25 cc. of water was put into the lungs by accident. This killed the animal almost instantly, apparently from paralysis of the heart.

Experiment III. Small dog, 2.5 kgm., was given 5 cc. furfural by stomach tube and this was washed in with 20 cc. water. The animal dropped to the floor in two minutes as if drunk, made some swimming movements, and groaned as if in pain. Vomiting occurred in four minutes with frothing at the mouth, and dyspnea followed. Death occurred in ten minutes after giving the furfural apparently from paralysis of the heart. A post mortem showed the heart was dilated, the lungs scarlet red in color and the blood contained bands of methemoglobin.

Experiment IV. The dog used weighed 9 kgm. It was given 2 cc. furfural by stomach tube with the results shown in the following protocol:

MINUTES	
0	Dog given 2 cc. furfural
6	Became ataxic and vomited
7	Urination, with trace of drug in urine ³
8	Vomited again; bile colored
22	Apparent improvement
30	Drowsy
75	Vomited a third time
92	Recovery complete after several hours

SUMMARY

1. The phenol coefficient of furfural, measured from its bactericidal action, is 0.26.

³ Joffroy and Serveaux (loc. cit., p. 4) injected a solution of 1:1,000 furfural, intravenously into a dog at a rate of 2.61 cc. per kilogram per minute, or 30.61 cc. per minute. In 259 minutes the animal excreted 4 liters of urine. Since, however, this was about one-half the volume injected it can not be considered a diuretic.

2. A 2 per cent solution of furfural entirely inhibits the action of yeast on dextrose. A 1 per cent solution delays only this fermentation. Weaker solutions have no effect.

3. Furfural is about one-half as toxic for gold fish as phenol, and about one-third as toxic as formaldehyde for the same animal.

4. Furfural in large doses has a paralytic effect on frogs similar to chloral. There are evidences of stimulation of the central nervous system when smaller doses are used but this is more marked in cats. Locally it is corrosive and anesthetic.

5. Furfural in increasing doses causes unsteady gait, inability to stand and finally paralysis in rabbits. Respiration is at first stimulated and irregular. Cyanosis is marked with larger doses. The reflexes may be increased at first and then decreased with larger doses. The fatal dose by stomach is about 0.8 cc. per kilogram of body weight.

6. The action of furfural on white mice is similar to that on rabbits.

7. When in 5 per cent solution, 0.6 cc. of actual furfural per kilogram of body weight causes only a slight drowsiness in cats; 0.12 cc. of the pure drug per kilogram causes increased irritability, increased respiration, salivation, and finally strychnine-like convulsions.

8. In dogs, the action is much the same as in cats, except that convulsions are elicited rarely. Respiration is paralyzed before the heart stops.

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THE ACTION OF CERTAIN DEPRESSANT DRUGS ON THE SENSORY THRESHOLD FOR FARADIC STIMULATION IN HUMAN SUBJECTS AND THE EFFECT OF TOBACCO SMOKING ON THIS ACTION

W. HALE AND G. P. GRABFIELD

*From the Department of Pharmacology in the Harvard Medical School,
Boston, Massachusetts*

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In view of the claims advanced for the quicker absorption rate of sodium barbital over barbital it was originally planned to study this by means of the effect of the sensory threshold for faradism as determined by the Martin (1) method. A priori, such claims would seem to be unfounded inasmuch as a very low concentration of hydrochloric acid, such as is found in the stomach contents, is sufficient to dissociate sodium barbital and precipitate it in the form of diethylbarbituric acid.

That the use of Martin's method of quantitative faradic stimulation should give results in such a study is based upon a number of quantitative studies using this method, or modifications of it, in determining its parallelism to certain variations in the response of human subjects to other stimuli and to its agreement in drug studies upon drugs such as acetphenetidine, alcohol and opium derivatives which have clear depressant effects (2). The exact significance of these observations, however, is somewhat uncertain since the method may not be so applicable to a quantitative study of depression in human subjects as it is to depression in nerve-muscle preparations against which the method originally was quantitated. The human organism is much more complex and introduces into a quantitative study such variables that only approximately accurate results may be expected. The variations in individual experiments is often so great that averages of a considerable number of separate experiments must be taken in

order to secure reasonable accuracy in the final results. The exact relation of variations in the threshold determined by this method to general nervous excitement or depression is not clear although the studies referred to above, both of physiological and of drug responses, suggest that it is a measure of a certain type of nervous tone at least.

We have followed Martin's technique, as reported by him and his pupils, as closely as possible, using non-polarizable electrodes of mercury, calomel, and salt solution and the first and second fingers of the left hand as test surfaces.

One of the several factors in the Martin method is the determination of tissue resistance. The method used is that described by Kohlrausch and by Martin, but we have experienced great difficulty in measuring this because of the lack of an area of tone silence in the Wheatstone bridge. It is accordingly possible that our figures for this factor are incorrect, due, we believe, to the fact that the tissues act as a condenser and have, therefore, an electrical capacity which we have been unable to eliminate. However, since readings with resistances of 10,000, 20,000, and 30,000 ohms in addition to the tissue resistance are made, it seems almost certain that the percentage error in estimating tissue resistance is small. The method cannot be said, however, to yield accurate results for single experiments but repeated observations probably yield valid averages.

With these factors in mind, we were prepared to find no demonstrable differences in the effects of barbital and barbital-sodium but it was a surprise to find that neither of these substances had more than the smallest, if indeed any, demonstrable effect on the threshold for faradic stimulation. Shortly thereafter an abstract of a study by Mendenhall (3) appeared in which he reported that tobacco smoking tended to bring this threshold to a normal figure whether the subjects were irritable or depressed. Since both the subjects in our experiments smoked during the experiments, parallel observations were made without smoking in order to find out whether tobacco had been responsible for our negative results with these drugs. Later antipyrine, acetphenetidine and acetylsalicylic acid were studied with and with-

out smoking to determine their effect upon this threshold and a very limited number of experiments were also made with phenyl barbital.

All the experiments were done in the morning, beginning at 9:30 or 10:00. The general procedure was as follows: A reading was made on each of the two subjects who then immediately took the drug with a full glass of water. Two or three readings at half-hour intervals were made after the ingestion of the drug. The breakfasts of the subjects were constant, one being very light and the other somewhat heavier. On days on which the effect of the drugs without the effect of tobacco was to be observed the subjects did not smoke from the night before until the end of the experiment. On days upon which the effect of smoking was to be measured both subjects smoked (pipe or cigarette) as constantly as possible, taking particular care to smoke while the readings were being made.

The results are recorded in the tables and plotted on the charts on a percentile basis derived from the minimum amount of faradic current necessary to cause sensation as measured in beta units. The beta unit is the average amount of faradic current required to cause minimal contraction of the frog's gastrocnemius stimulated through the sciatic nerve in the nerve-muscle preparation. The original reading was considered as 100 per cent and the variations from this in the subsequent readings were calculated in terms of per cent. Controls without the ingestion of drugs were made on both subjects with and without smoking. The normal curves correspond in a general way to the curves reported for the diurnal variations at this time of the day.

The diethylbarbituric acid type of sedative shows very slight effect on this threshold not only in the smoking but also in non-smoking experiments (fig. 1). In this group we found that the curves resulting from sodium barbital and barbital were so similar that we have plotted the average of the observations together. The average dose in the case of the experiments with smoking was 0.41 gram and without smoking 0.43 gram. The weight of the two subjects was quite different but an equal num-

ber of observations on each is included in each of these series of experiments. A glance at the curve will show that none of the four curves fall outside of the 10 per cent variation which is the error inherent in the method. However, it is evident that barbital without the additional factor of tobacco tended to change the type of curve whereas this effect was nullified in smoking experi-

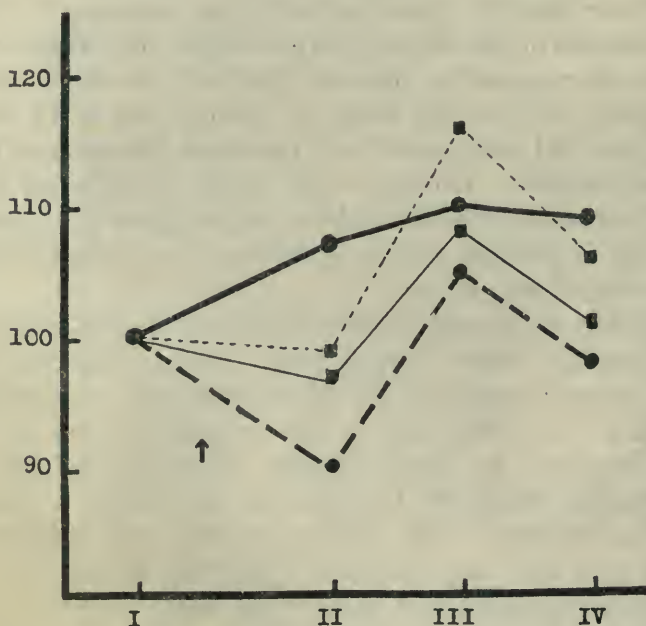


FIG. 1. Solid line, diethylbarbituric acid hypnotics without smoking; broken line, diethylbarbituric acid hypnotics with smoking; light solid line, control without smoking; dotted line, control with smoking.

ments, in which the results approach the normal curve with surprising regularity. In many of these experiments the subjects distinctly noted subjective effects from the use of the drugs. A few experiments with phenobarbital yielded no greater effect on this threshold than those shown in figures, although in this case doses were used sufficient to produce symptoms of giddiness; in several instances the subjects actually dozed between readings.

In the case of antipyrine and acetphenetidine the effects are much more striking. Instead of the usual finding of a rise in irritability amounting to 2 or 3 per cent at the end of the first half-hour we found after antipyrine an average fall of irritability of nearly 30 per cent, providing the subjects were not smoking.

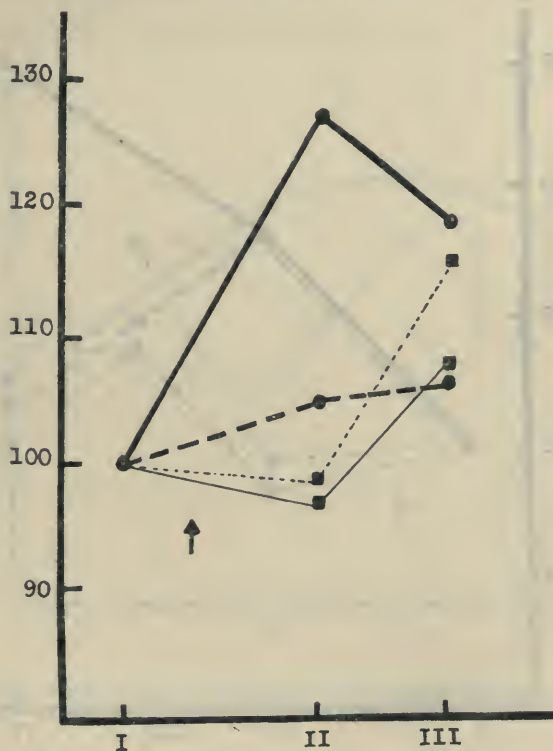


FIG. 2. Solid line, antipyrine without smoking; broken line, antipyrine with smoking; light solid line, control without smoking; dotted line, control with smoking.

In the smoking experiments this fall is reduced to 5 per cent. Apparently the effect of antipyrine on this threshold begins to wear off in about an hour (fig. 2).

In the case of acetphenetidine (fig. 3) the rise is more continuous and more prolonged. Martin, Grace and MacGuire (2) found an average lowering of irritability amounting to 20 per cent

one hour after the ingestion of acetphenetidine. This observation plotted by our method falls on our curve which shows at the end of one hour and a half a rise in the threshold of 32 per cent. In the smoking experiments it was found that the initial effect of acetphenetidine could be traced but that smoking apparently

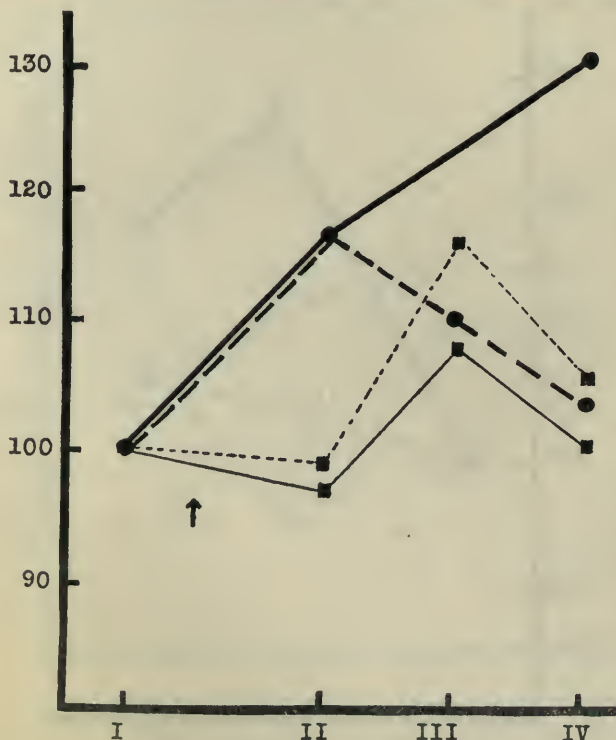


FIG. 3. Solid line, acetphenetidine without smoking; broken line, acetphenetidine with smoking; dotted line, control with smoking; light solid line, control without smoking.

brings the curve of irritability within normal limits after the first half-hour. The differences in the attainment of maximum effect in the case of antipyrine and acetphenetidine is probably explained by the fact that the latter is converted into p-amidophenol before this effect is attained, whereas the former acts directly upon absorption.

We believe that it is evident from the preceding that tobacco smoking is antagonistic to the action of these drugs upon this threshold. A clearer idea of this effect may be seen when all of the smoking and all of the non-smoking experiments are averaged and the respective curves plotted (fig. 4). The number of these observations is such that we believe a variation of 5 per cent may be considered significant.

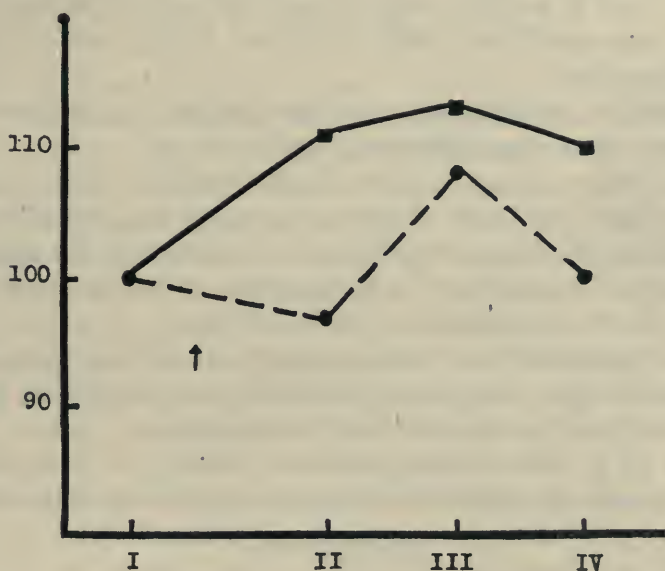


FIG. 4. Solid line, all experiments without smoking; broken line, all experiments smoking.

These observations do not prove that tobacco smoking antagonizes the action of these drugs in all of their actions. The exact threshold which is being determined when the skin is stimulated by a faradic current is still not known, nor is the path which these impulses take to the brain certain. Some evidence has been offered by one of us (4) to show that these are carried in the same path as the thermal fibers and it is perhaps significant that the drugs which produce the most pronounced effects upon this threshold both belong to the antipyretic group. The tobacco

antagonism may be, therefore, limited to a certain group of cells and may not be a general effect, but the evidence suggest that its action is more diffuse than that of any one of the drugs studied and it is possible that these experiments may be of therapeutic significance.

SUMMARY AND CONCLUSIONS

1. The effect of the diethylbarbituric acid hypnotics, of antipyrine, and of acetphenetidine upon the sensory threshold for faradic stimulation was measured in two human subjects by the Martin method.

2. Parallel observations were made when the subjects were smoking and during abstinence from smoking.

3. In non-smoking experiments the barbitals were found to have a slight transitory effect in decreasing the irritability.

4. Antipyrine in non-smoking experiments showed a maximum effect within one-half hour after ingestion in the manifestation of nearly 30 per cent decrease in irritability.

5. Acetphenetidine showed a slightly greater maximum effect than antipyrine, more slowly attained, and reached at the expiration of one hour and a half after ingestion.

6. In all 3 cases smoking tended to nullify the effect of these drugs bringing the threshold observations within normal limits.

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ON THE BIOLOGICAL SIGNIFICANCE OF LIPOIDS THE ACTION OF KEPHALIN AND LECITHIN

W. STORM VAN LEEUWEN AND A. V. SZENT GYÖRGYI

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Chemical research work of recent years has led to a classification of the lipoids whereby they are separated into two distinct groups, the phosphatides and the cerebrosides. The phosphatides are subdivided by means of the number of nitrogen and phosphorus atoms of their moleculars. The best known group of phosphatides which have attracted the interest of physiologists most, seems to be the group of the monoamino-monophosphatides, formed by kephalin and lecithin substances.

These monoamino-monophosphatides seem to belong to the normal constituents of nearly every living cell, and as they are closely related chemically it is very difficult to separate them quantitatively. This may be the reason why in many experiments which have been undertaken to study the physiological properties of these lipoids no guarantee could be given as to the purity of the material used. Many investigators have studied the behavior of lecithin, but as we know from the recent work of Levene and others, that it is extraordinarily difficult to obtain lecithin in absolutely pure form, it may be taken for granted that in all these researches the lecithin used, was to a more or less degree contaminated with kephalin, and conversely in those experiments where the action of kephalin has been studied the material used will have been as a rule contaminated with lecithin. So it is known for instance from the analytical data given by Koch and Woods (1) fifteen years ago, that some of the so-called commercial lecithin preparations contained more kephalin than lecithin, still in biochemical literature of to-day it is a common error to find the amount of lipid soluble phosphorous of a substance to be identified with the "lecithin" content.

Since we found in former investigations (2) that though lecithin and kephalin are closely related, some of their physiological properties (i.e., augmentor influence on drug action) may differ considerably, we deemed it of interest to extend this research to other physiological actions of lecithin.

We have found in earlier experiments, that lecithin, when injected intravenously into an animal produces a very marked fall of blood pressure.

Clark (3) has shown that an isolated frog heart, which by long continued perfusion has been rendered hypodynamic, can be brought into an almost normal condition by the action of lecithin. Landsteiner and Jagic (4) have found that the hemolytic action of a colloidal solution of silicic acid is greatly increased by the action of lecithin.

Finally it is known that the action of cobra poison can be "activated" by the addition of lecithin.

For reasons stated above in all these cases it is improbable that the lecithin used by the different investigators has been pure and so it remained an open question whether the physiological actions of lecithin were to be ascribed to the lecithin or to the kephalin content of the preparation used.

In the present publication we have tried to settle this question. The work described here has been made possible for us by the courtesy of Dr. Levene who kindly provided us with very pure samples of lecithin and kephalin and with mixtures of known amounts of lecithin and kephalin.

The analytical data of our lecithin and kephalin samples as given by Levene are as follows:

<i>Lecithin sample R. 324</i>			<i>Kephalin sample R. 294</i>		
C.....	65.48		C.....	63.86	
H.....	11.07	amino N 0	H.....	9.48	amino N 100
N.....	1.93		N.....	1.66	Total N 100
P.....	3.58		P.....	3.16	

I. ISOLATED GUT EXPERIMENT

The results of our investigations on the influence of phosphatides on the action of drugs on isolated intestine, which formed

the starting point of our further inquiries into the physiological action of lipoids, are published elsewhere (5). Here we only want to recapitulate the chief results of the work.

In the first place we were able to show that kephalin has a strong augmentor influence on the action of pilocarpine on the isolated gut. Lecithin had only a slight action or no action at all in this respect. If mixtures of kephalin and lecithin emulsions were added to the gut, the augmentor action of the kephalin was present. But if an emulsion was prepared from a mixture of kephalin and lecithin (mixing them in ethereal solution) the action of kephalin was decidedly weakened, and became very inconstant, even being entirely absent in some cases. In this way it was demonstrated not only that the action of kephalin on the isolated gut differs from that of lecithin but that, the action of kephalin can be inhibited by the lecithin.

II. BLOOD PRESSURE EXPERIMENT

In earlier experiments working with commercial lecithin preparations, intravenous injections of lecithin were always followed by a deep fall of blood pressure, very similar to that seen in anaphylactic or histamine shock. We now repeated these experiments with chemically pure preparations, to decide whether this action is characteristic of lecithin, or was due to the impurities of our former preparations in the first place to their content of kephalin.

For this purpose cats were narcotized with ether and artificial respiration was introduced. The blood pressure was recorded from the carotid with a mercury manometer and the colloids to be tested, were injected in the vena femoralis. All injections were made in twenty-seconds with a small record syringe. The colloidal solutions were always filtered through paper filters before being injected. The following figures give instances of the effect of these injections.

In figure 1a, 2 cc. of a 0.5 per cent emulsion of lecithin were injected. Figure 1b shows the effect of 1 ccm. of a 0.25 per cent kephalin in emulsion. In figure 1c, twice 1 ccm. of the ultrafiltrate of this kephalin in emulsion was introduced. This figure

shows that the effect of kephalin cannot be ascribed to non colloidal decomposition products of this colloid. This experiment was repeated three times with the same result.

Intravenous injection of lecithin is not followed by any typical alteration of blood pressure. Injection of kephalin is followed by a deep fall of blood pressure.

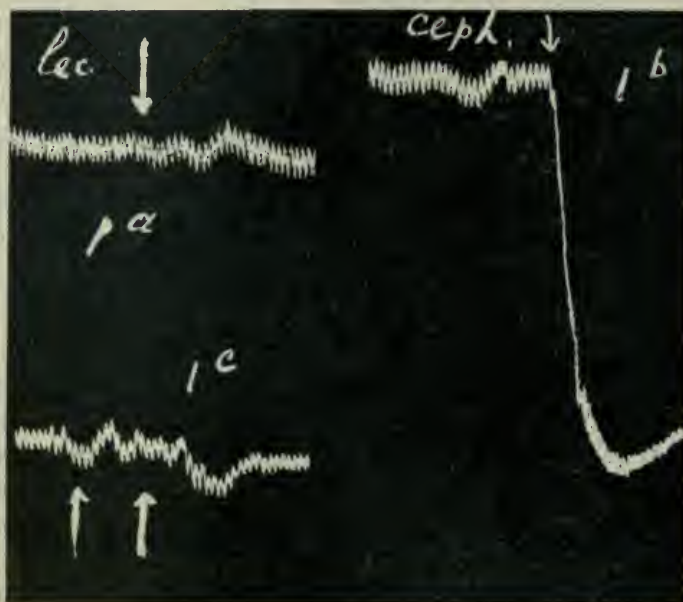


FIG. 1. INFLUENCE OF LECITHIN AND KEPHALIN ON BLOOD PRESSURE

1a, at injection of 2 cc. of 0.5 per cent emulsion of lecithin; no action.

1b, injection of 1 cc. of 0.25 per cent emulsion of kephalin; very strong fall of blood pressure.

1c, at injection of ultrafiltrate of kephalin; no action.

III. ACTION ON THE HYPODYNAMIC FROG HEART

It is known from the experiments of Clark that the frog heart after having been perfused for several hours with a physiological salt solution enters into a "hypodynamic" state in which the rate and the force of the contractions are greatly reduced. Through the perfusion of serum the action of the heart can be greatly improved, and it has also been shown by Clark (6) that

this action of serum was due to its lipid fractions. Of the lipoids examined lecithin seemed to be very active in this respect. We repeated Clark's experiments with chemically pure preparations of kephalin and lecithin. Our results are demonstrated by the curves of figure 2, a to e. The same results were obtained in other experiments.

Figure 2a shows the heart freshly isolated; figure 2b, after a perfusion of twenty-four hours; figure 2c, after 2 cc. of 0.5 per cent of lecithin was added to the perfusing solution. This injection produced no effect. Only a much greater amount of lecithin slightly improved the condition of the heart in other experiments. Figure 2d shows the heart after the addition of



FIG. 2. INFLUENCE OF LECITHIN AND KEPHALIN ON THE HYPODYNAMIC FROG HEART

- 1a, heart freshly isolated.
- 1b, after twenty-four hours perfusion.
- 1c, after injection of lecithin.
- 1d, after injection of ultrafiltrate of kephalin.
- 1e, after injection of emulsion of kephalin.

1 cc. of 0.25 per cent kephalin ultrafiltrate; figure 2e after the addition of 1 cc. of the kephalin emulsion. The heart is restituted to its original efficiency.

Lecithin has practically no effect on the hypodynamic frog heart. Kephalin has a very strong improving action, which is not due to non colloidal decomposition products.

IV. AUGMENTATION OF THE ACTION OF COLLOIDAL SILICIC ACID

When a colloidal solution of silicic acid is added to an emulsion of red blood cells it has a double action, i.e., the blood cells are agglutinated and frequently there also occurs slight hemolysis. Both these actions of silicic acid can be augmented by lecithin.

1. *Hemolytic action*

It was first described by Landsteiner and Jagic (7) that the hemolytic action of colloidal silicic acid can be increased to a very considerable degree by the addition of slight amounts of lecithin. This observation has become important as through it an analogy with serological hemolysis was offered. Since at the time when Landsteiner and Jagic performed their experiments it was not known that nearly all lecithin preparations contain kephalin and since it is unlikely that they had at their disposal samples of lecithin of the same purity as the one we have obtained from Dr. Levene we thought it worth investigating whether the augmentor action of lecithin on silicic acid hemolysis was due to the lecithin or to impurities, i.e., kephalin or other compounds.

The following experiments were performed. In small test tubes three identical series of various dilutions of colloidal silicic acid were set out. The silicic acid solution had been prepared by treating 2 cc. of silicium-aethylester in 100 cc. of distilled water after addition of 1 drop of n. HCl for four hours on a water-bath with reflux condenser.

The first test tube of every series contained 1 cc. of a 2 per cent solution of silicic acid, the second tube 1 cc. of a 1 per cent solution, the third tube 1 cc. of a 0.5 per cent solution and so forth. The last tube (no. 12) contained only water. All the solutions were rendered isotonic with blood by the addition of salt solution (NaCl). Now to every tube of the first series 0.2 cc. of 0.9 per cent NaCl was added; to every tube to the second series 0.2 cc. of 0.25 per cent of an emulsion of lecithin in isotonic NaCl, and to every tube of the third series we added 0.2 cc. of 0.25 per cent kephalin. In this experiment we worked with a kephalin sample prepared in this laboratory since the samples obtained from Levene, after having been kept in our laboratory for a year seemed to be partly decomposed and gave per se a marked hemolysis.

It must be stated that our kephalin sample though prepared with as much care as was possible was not in the least as pure as Levene's sample and certainly contained impurities. We were

sure however that it contained a large percentage of kephalin and on examination it produced no hemolysis. Had it done so it would have been uncertain whether this action was due to the kephalin or to contamination with lecithin. Since we found that our kephalin samples had no activating action we feel justified in considering the experiment to be conclusive even though our kephalin was not pure. Our lecithin sample however had to be absolutely pure to permit any conclusion to be drawn. Therefore Levene's pure lecithin was used.

The results of this experiment are given below.

	NUMBER OF TEST TUBE											
	1	2	3	4	5	6	7	8	9	10	11	12
I. Control.....	0	0	0	0	0	0	0	0	0	0	0	0
II. Lecithin.....	0	0	+	+	+	+	+	+	+	+	(+)	0
III. Kephalin.....	0	0	0	0	0	0	0	0	0	0	0	0

Complete hemolysis is marked with +. Incomplete hemolysis with (+).

Thus we found in concordance with Landsteiner and Jagic that lecithin activates silicic acid in rendering it hemolytic. Kephalin has no marked activating action.

2. Agglutination

As has been stated above, colloidal solutions of silicic acid do not only have a hemolytic action but they also cause agglutination of red blood corpuscles and it seemed to us of interest to investigate whether this last mentioned action could also be influenced by lipoids. To test this question we performed the following experiments.

Three series of various dilutions of colloidal silicic acid were prepared in the same manner as in the hemolysis experiments described above. All the test tubes contained 1 cc. of silicic acid solution but the concentrations varied so that each tube contained twice the amount of silicic acid as the next one. The last tube (no. 12) contained 1 cc. of aqua dest. only. To every tube was added 0.2 cc. of an 0.1 mcl. phosphate buffer of pH 7 and 0.2 cc. of a 0.9 per cent NaCl solution. Then to the tubes of the

first series 0.2 cc. of water was added, to the tubes of the second series 0.2 cc. of 0.25 per cent lecithin and to the tubes of the third series 0.2 of 0.25 per cent kephalin. The tubes were then shaken and after 5 minutes to every tube was added 0.5 cc. of 5 per cent washed red blood corpuscles of the cat (in isotonic salt solution).

Three readings of agglutination were made, one immediately after the addition of the blood, one after the series had been kept at room temperature for twenty minutes and the third reading after keeping them at 37°C. for thirty minutes.

The results of this experiment are given in the table below.

	NUMBER OF TEST TUBE											
	1	2	3	4	5	6	7	8	9	10	11	12
I. Control.....	-	-	-	-	-	-	-	-	-	-	-	-
II. Lecithin.....	-	-	-	++	-	-	-	-	-	-	-	-
III. Kephalin.....	-	-	-	-	-	-	-	-	-	-	-	-
I. Control.....	-	-	-	-	-	-	-	-	-	-	-	-
II. Lecithin.....	-	-	+	+++	+	-	-	-	-	-	-	-
III. Kephalin.....	-	-	-	-	-	-	-	-	-	-	-	-
I. Control.....	-	-	-	++	++	+	+	+	+	-	-	-
II. Lecithin.....	-	+	++	+++	+	-	-	-	-	-	-	-
III. Kephahlin.....	-	-	-	-	-	-	+	+	+	+	-	-

Complete agglutination is indicated by +++. Weak agglutination is indicated by ++ and +. Very slight agglutination is not noted.

It is clear from this table that lecithin is able to quicken the agglutinating action of certain concentrations of silicic acid, in other concentrations it seems to inhibit this action. Kephalin has a slight inhibiting action on silicic acid agglutination.

Since it cannot be doubted that lecithin activates silicic acid whereas kephalin does not, it seemed of interest to know whether silicic acid has a direct action on some lipoids as this might lead to finding an explanation for the mechanism of silicic acid agglutination. We therefore repeated the experiment described above, without adding red blood corpuscles and paid attention to changes in the various silicic acid solutions after addition of lipoids.

In the kephalin series no marked change could be observed. In all tubes the liquids were slightly turbid, just as the control-tubes, containing only water and kephalin and no silicic acid. The reverse was seen by lecithin. On adding the lipid in the first six tubes immediately a strong turbidity occurred. We denoted the turbidity by +, the precipitation by ×.

Immediately after adding the lecithin the following reading was made.

1	2	3	4	5	6	7	8	9	10	11	12
+	+	+	++	++	+	--	--	-	-	-	-

Five minutes later:

+	+	+	++	××	+	--	-	-	-	-	-
---	---	---	----	----	---	----	---	---	---	---	---

As can be seen the reaction has a distinct maximum, just as one is accustomed to see in other colloidal reactions. The relation of the amounts of silicic acid and lecithin in the tube which showed the greatest optical turbidity was 1.25:6.5.

In this experiment then was shown that silicic acid precipitates lecithin, while kephalin is not influenced.

The next point to investigate was whether lecithin in augmenting agglutination by silicic acid has a direct action on the red blood corpuscles. To decide the question the following experiment was performed.

Three series of silicic acid dilutions were prepared as described above, every tube containing 1 cc. of colloidal silicic acid of various dilutions. To every tube 2 cc. of 0.9 per cent NaCl dissolved in 0.1 mol. $\frac{\text{prim. phosphat}}{\text{secund. phosphat}}$ pH = 7 buffer was added.

Moreover in the first series 0.2 cc. of 0.9 per cent NaCl was added to every tube. In the second series we added 0.2 cc. 0.1 per cent lecithin in 0.9 per cent NaCl. To the third series nothing was added.

All the tubes were kept for half an hour at 37°C. Then to the first and second series in every tube 0.5 cc. of 5 per cent washed

blood was added. In the third series we added to every tube 0.7 cc. of a mixture of lecithin with blood obtained by mixing 5 cc. of 5 per cents washed red cells with 0.1 per cent of lecithin solution in 0.9 per cent NaCl. This mixture was also kept at 37°C. for half an hour.

In this way we had three series, the first containing no lipid, the second containing lecithin which had been in contact with the silicic acid for half an hour, while the third series contained lecithin which had been in contact with red blood corpuscles for half an hour and had had time to combine with the latter.

Three readings of agglutination were made: One two minutes after the addition of blood, one twenty minutes later, the third reading after the tests were left half an hour at 37°.

	NUMBER OF TEST TUBE											
	1	2	3	4	5	6	7	8	9	10	11	12
I	—	—	—	—	—	—	—	—	—	—	—	—
II	—	—	—	—	+	+	—	—	—	—	—	—
III	+	—	—	—	—	—	—	—	—	—	—	—
I	—	—	—	—	—	—	—	—	—	—	—	—
II	—	—	—	—	+	+	—	—	—	—	—	—
III	+	+	+	+++	+++	+++	—	—	—	—	—	—
I	—	—	—	—	—	+	+	+	+	—	—	—
II	—	—	—	+	++	—	—	—	—	—	—	—
III	+++	+++	+++	+++	+++	—	—	—	—	—	—	—

It is obvious then from this experiment that lecithin has the strongest action if it has been in contact with the red blood corpuscles before, so that it has had time to combine with them.

V. ACTIVATION OF COBRA VENOM

After preliminary work by Flexner and Noguchi (8), it was shown by Kyes (9), that the hemolytic action of cobra venom can be augmented by lecithin. Experiments performed with kephalin showed that this substance has the same augmentor property.

The following experiments were undertaken.

Four series of various dilutions of cobra venom in 0.9 per cent salt solution were prepared. The first test tube of every series contained 1 cc. of 0.1 per cent cobra venom solution, the second tube contained 1 cc. of 0.05 per cent cobra venom, the third tube 1 cc. of 0.025 per cent cobra venom and so forth. The last tube of every series contained physiological salt solution only.

To every tube of the first series 0.2 cc. of 0.9 per cent salt solution was added; to every tube of the second series 0.2 cc. of a 0.25 per cent emulsion of lecithin in physiological salt solution; to every tube of the third series 0.2 cc. of 0.25 per cent of kephalin emulsion was added, whereas to every tube of the fourth series 0.2 cc. of an emulsion contained 0.25 per cent lecithin, and 0.25

TABLE 1
Hemolysis of red blood corpuscles of the cat

	MILLIGRAM COBRA VENOM												
	1	0.5	0.2	0.1	0.05	0.025	0.012	0.006	0.003	0.0015	0.0007	0.0003	0
Control.....	+	-	-	-	-	-	-	-	-	-	-	-	-
Lecithin.....	+	+	+	+	+	+	+	+	+	+	-	-	-
Kephalin....	+	+	+	+	+	+	-	-	-	-	-	-	-
Lecithin and kephalin....	+	+	+	+	+	+	+	+	-	-	-	-	-

per cent of kephalin was given. This lecithin-kephalin mixture was prepared by first mixing a solution of lecithin and of kephalin in ether and subsequently pouring out the mixture in water from which the ether was expelled by evaporation.

All the tubes were shaken and then to every tube 0.2 cc. of 0.5 per cent suspension of washed red blood corpuscles of the cat were added.

Readings of hemolysis were made after the tubes had been kept at 37° for one hour. The results are given in table 1.

This table demonstrates, that kephalin emulsions are able to activate cobra venom, though in a smaller degree than lecithin does. When a mixture of lecithin and kephalin is given, the activation of lecithin seems to be inhibited partly by kephalin.

This experiment was repeated one week later with the same stock emulsion of lecithin, kephalin and lecithin-kephalin, which had been used in the first experiment; this time however three readings were made, the first one after the tube had been kept for ten minutes at room temperature, the second reading after twenty minutes at room temperature, the third one after one hour at 37°C. The results of the various readings are given in table 2. The difference between lecithin and kephalin actions is also clearly shown in this table.

TABLE 2

	0.25	0.12	0.06	0.03	0.015	0.002	0.004	0.002	0.001	0.0005	0
Control.....	—	—	—	—	—	—	—	—	—	—	—
Lecithin.....	+	+	+	—	—	—	—	—	—	—	—
Kephalin.....	—	—	—	—	—	—	—	—	—	—	—
Lecithin and kephalin....	—	—	—	—	—	—	—	—	—	—	—
Control.....	—	—	—	—	—	—	—	—	—	—	—
Lecithin.....	+	+	+	+	+	—	—	—	—	—	—
Kephalin.....	—	—	—	—	—	—	—	—	—	—	—
Lecithin and kephalin....	—	—	—	—	—	—	—	—	—	—	—
Control.....	+	+	—	—	—	—	—	—	—	—	—
Lecithin.....	+	+	+	+	+	+	+	+	—	—	—
Kephalin.....	+	+	+	+	—	—	—	—	—	—	—
Lecithin and kephalin....	+	+	—	—	—	—	—	—	—	—	—

In this experiment kephalin gave per se marked hemolysis, which being not complete, is not indicated in the tables, but still disturbed the reading. This hemolytic effect was completely inhibited by the lecithin, present in the fourth series. The kephalin used in this experiment was a very pure sample, being absolutely lecithin free. However it had been kept in our laboratory for more than two years and had deteriorated to a certain degree, so that it had an acid reaction. We thought it possible, that the hemolytic action of kephalin might be due to decomposition products and therefore we made another experiment similar to those mentioned above, but using in this case a sample of kephalin, which we prepared in our own laboratory and which, although

certainly not pure, contained some kephalin, which had not undergone an appreciable deterioration. This preparation had per se no hemolytic action. The results of this experiment are given in table 3.

TABLE 3

	READING AFTER ONE HOUR AT 37°											
	0.25	0.12	0.06	0.03	0.015	0.008	0.007	0.002	0.001	0.0005	0.00025	0
Control.....	—	—	—	—	—	—	—	—	—	—	—	—
Lecithin.....	+	+	+	+	+	+	+	+	+	+	—	—
Kephalin.....	+	+	+	+	+	—	—	—	—	—	—	—

It is clear, that our kephalin preparation also was able to increase the action of cobra venom. Lecithin was, as in the former experiments, much more active in this respect. The difference in the action of lecithin and kephalin is most clearly demonstrated by the following experiment, in which the quantity of cobra venom was kept constant and the quantity of lipoids varied. The figures in this table give the absolute quantity of lecithin in milligrams.

Every tube contains 0.02 mgm. of cobra venom in 1 cc. 0.9 per cent NaCl plus 0.2 cc. of 5 per cent washed red blood corpuscles of the cat, plus 0.2 cc. of the lipid emulsion. Reading after one hour at 37°. Only complete hemolysis is noted. Kephalin from Levene was used.

	0.5	0.25	0.12	0.06	0.03	0.015	0.007	0.004	0
Lecithin.....	+	+	+	+	+	+	—	—	—
Kephalin.....	—	—	—	—	—	—	—	—	—

SUMMARY

Cobra hemolysis can be increased not only by lecithin, but also by kephalin. The latter lipid however is less active in this respect than lecithin.

It is known from researches, chiefly performed by Ludecke (10), Manwaring (11), Delezenne, and Fourneau (12), that lecithin can be decomposed by the action of cobra venom by which pro-

cedure a mono-fatty-acid-lecithin is formed. This so called lecithide is responsible for the hemolytic action of cobra venom-lecithin mixtures. It is probable, that the hemolytic action of cobra venom-kephalin mixtures depends on a similar mechanism, but we were not in a position to decide the matter. We wish, however, to record some observations made in the course of our experiments. Firstly we noted that the action of cobra venom on kephalin resulted in the formation of an acid, depending on the duration of the action. The acid production could be followed by titration. When cobra venom-kephalin mixtures were kept at 37°C, during twenty-four hours, the mixture became soluble in alcohol and acetone. Hence it is probable that the kephalin was decomposed by the action of cobra venom.

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ON THE SENSITIVITY OF DIFFERENT NERVE ENDINGS TO ATROPINE

V. E. HENDERSON

Laboratory of Pharmacology, University of Toronto

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Pharmacological textbooks state that atropine depresses or very often paralyzes nerve endings and would leave the clinician to infer that this action occurred with what may be described as therapeutic doses. As is well known this is not the case. The normal therapeutic dose of atropine slows the heart centrally; the vagus endings are not paralyzed. It seemed, therefore, worth while to attempt to ascertain the amounts of atropine necessary to cause so complete a depression of the various nerve endings of the bulbosacral autonomic outflow (parasympathetic) that nerve stimulation with an induced interrupted current would be ineffective.

In the course of this study several technical difficulties appeared. First, very considerable variations in the effective dose for any ending were found from animal to animal which did not correspond with weight variations. Second, small doses given at such intervals as were necessary to testing the various endings under examination did not seem to give a true picture of the effect of the same quantity administered as one dose. Consequently, to obtain comparisons between one ending and others the plan was adopted to study as a rule three endings in each experiment. This entailed frequently very extensive operations with a corresponding increase in experimental failures.

Table 1 sets forth in brief the results obtained. From the sequence shown, there was in no experiment a departure; by this is meant that vagus stimulation always became ineffective with smaller doses than chorda, this again smaller than intestinal vagus and this than the sacral supply to the bladder, etc.

TABLE 1

ENDING	SMALLEST EFFECTIVE DOSE				LARGEST EFFECTIVE DOSE			
	Animal	Num-ber	Weight <i>kgm.</i>	Amount of dose. <i>mgm.</i>	Animal	Num-ber	Weight <i>kgm.</i>	Amount of dose <i>mgm.</i>
Cardiac vagus	Dog	9	15	0.1	Dog	13	13	1.0
Chorda secretory	Dog	18	21	0.5	Dog	16	14.3	2.5
Chorda vasodilator	Dog	18	21	20	Dog	16	14.3	40
Vagus intestine rhythmic	Dog	20	10.5	1.0	Dog	22	16	25 Little
Vagus peristalsis	Dog	15	16.2	0.5	Dog	25	8.2	50 No effect
Sacral to bladder tonus	Dog	20	10	None after 1.0	Dog	17	8	30 Questionable peris- talsis
Sacral to bladder contraction	In all cases equal after 50 mgm., after 100 very slightly weakened				None after 0.5			
Cardiac vagus	Cat	7	4.2	0.25	Cat	6	4.3	0.3
Intestinal vagus peristalsis	Cat	12	3.6	0.25 Lower tonus but peristalsis	Cat	7	4.2	0.25 Lowered tonus
				10.0 Slight if any, rapid to pinching				30 No peristalsis save on pinching
Sacral to bladder	Cat	Tonus decreased by 0.25 to 1 mgm., contractions not affected by 50. 200 mgm. caused no weakening in one case						

TABLE 2

Average dose of atropine just producing demonstrable effect in dogs of 8 to 10 kgm.

1. 0.1 mgm:	Chorda secretion decreased from 15 to 10 drops
2. 0.2 mgm.	Cardiac vagus less effective
0.5 mgm.	Heart rate increased and vagus ineffective
3. 0.1 to 0.2 mgm.	Distinct decrease in tonus of pylorus, and of small intestine
4. 0.2 mgm.	Decrease in bladder tonus, absent with 0.3 mgm.
5. 0.5 mgm.	Pupil dilated slightly reacts to light and accommodation
0.6 mgm.	Dilation almost maximal
6. 0.7 mgm.	Slight decrease in blood flow from salivary gland on chorda stimulation as compared with before atropine
7. 30 mgm.	Vagus stimulation produces little, if any, increase in rhythmic movements, peristalsis slight or absent
8. 100 mgm.	No decrease in the isotonic or isometric contractions of the bladder on sacral stimulation

The experiments which dealt with the effect on intestinal vagus and sacral roots to the urinary bladder and rectum will be reported in full in another paper. In these it was, however, noted that very small doses depressed the existing tonus, if any, of the organ. This, in the case of the intestine, very greatly decreased the extent of the response of the intestine to stimulation of the vagus because the movements, rhythmic or peristaltic, were more shallow or of less force and produced less disturbance of the recording mechanism. They might indeed have been overlooked in some cases had the apparatus employed not been a delicate one. The bladder, however, continued to give responses either isotonic or isometric equal to those before the administration of such large doses as 100 mgm. to a dog or even to a cat although its tone was decreased by even a small dose.

It may be noted that the observations on the intestine confirm those of Bayliss and Starling (1), those on the bladder those of Langley and Anderson (2) and of Edmunds and Roth (3), and those on the salivary gland of Loewi and Henderson (4).

In therapeutics, however, what is required very often is not an ineffective ending but one that has been depressed in its action. A thorough quantitative study of these effects has not as yet been made but incidentally the results noted have been condensed in table 2.

Several studies have been made in man. In V. E. H., weight 65.4 kgm., 0.2 mgm. causes a decrease in nasal secretion but not marked drying of the mouth, while 0.3 mgm. dries the mouth. This dose will also relieve griping produced by aloes. None of these doses seem to affect the pupil. Two milligrams are necessary to give an increase in cardiac rate to 150 and in this case the pupil is dilated but not maximally.

SUMMARY

1. The endings of the bulbosacral autonomic outflow are rendered ineffective by atropine in the following order: cardiac vagus, chorda secretor, chorda vasodilator, intestinal vagus, bladder. The latter is not affected.

2. The endings are depressed in the following order: nasal, chorda secretory, cardiac vagus, tonus of pyloric sphincter, and small intestine, bladder, oculomotor to pupil, salivary vasodilator, vagus to intestine for rhythmic and peristaltic movements.

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ON THE ACTIVE PRINCIPLES OF THE PITUITARY GLAND

HAROLD WARD DUDLEY

From the Department of Biochemistry and Pharmacology, National Institute for Medical Research, London

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In a previous paper (1) it was shown that the oxytocic principle of the pituitary gland could be extracted, without appreciable loss, from aqueous solution, by butyl alcohol boiling under reduced pressure. By this method about 50 per cent of the total solid matter of the aqueous extract of dry infundibular substance can be eliminated, and the active residue, after removal of the butyl alcohol, is a pale yellow, sticky, hygroscopic, partly crystalline material. The process seemed to be sufficiently promising to warrant the working up of a considerable quantity of dry pituitary powder in the hope of making further progress toward the isolation of the uterine stimulant.

About 20 grams of the semi-crystalline butyl alcohol extract were obtained from 600 grams of dry infundibular substance, and on the general assumption that the active principle is basic in character a variety of attempts at isolation were made.

At the outset it was realized that the number of methods available was strictly limited by the unstable nature of the substance in question. It is known from the work of Guggenheim (2) that it is rapidly destroyed by normal sodium hydroxide in the cold, and although stable to sodium carbonate under similar conditions it was found during this work that quite mild treatment with this reagent is sufficient to bring about its decomposition. It is relatively more stable to acids, but is easily destroyed by them as Abel and Nagayama (3) have demonstrated, boiling for thirty minutes with 0.5 per cent hydrochloric acid resulting in its almost complete disappearance. It is also very readily

adsorbed on to precipitates which may be formed in its presence, such as lead sulphide (2). In the course of these experiments it was found that the precipitation of mercuric sulphide in a solution containing the oxytocic principle removed the latter completely. That hydrogen sulphide itself had no deleterious action was shown in a control experiment. The active principle is adsorbed from solution even by such indifferent substances as talcum (2). Attempts to remove the substance from these adsorbents by continuous washing with various solvents met with no success.

It is thus apparent that no process of purification involving either the use of alkalis, (e.g., the decomposition of phosphotungstates with baryta) or the production of precipitates of the sulphides of the heavy metals (e.g., the decomposition of mercury salts) is likely to be employed successfully in the isolation of the pituitary uterine stimulant.

The aqueous solution of the butyl alcohol extract, containing the oxytocic and pressor principles, gave the following positive reactions: Millon (tyrosine), glyoxylic acid and bromine (tryptophane), Molisch (carbohydrate) and Pauly (iminazole). The biuret reaction was doubtful, at any rate, very weak. The solution gave a slight amorphous precipitate with H_2PtCl_6 , heavy precipitates with HAuCl_4 , HgCl_2 and phosphotungstic acid and a sticky precipitate with picric acid.

It was evidently a very complex mixture. It was found that the addition of alcohol to the aqueous solution caused the deposition of granular crystalline material of a non-hygroscopic nature possessing very little activity on either the blood pressure or the uterus. On increasing the strength of the alcohol further fractions of similar material were obtained, but these brought down with them a considerable amount of activity. However, the final fraction, which was almost completely soluble in absolute alcohol and which could be precipitated practically completely from this solvent by the addition of dry ether, was a yellow, very hygroscopic powder of great activity. The activity tended to concentrate in this fraction which was used in attempts to effect further purification.

Even if gold or mercury salts of the oxytocic principle could be obtained they would be useless for purposes of isolation since their decomposition would involve the removal of the metal by means of H_2S with consequent disappearance of activity, and as the quantitative physiological test (isolated uterus) is the only guide to the presence of the substance sought for no progress could be made by such means. A platinum salt, however, can be decomposed by double decomposition with potassium chloride, so that if such a salt of the oxytocic principle could be obtained it would seem possible to decompose it without loss. This possible method was tried in aqueous and also in alcoholic solution, but without much success. A precipitate was obtained on adding an alcoholic solution of H_2PtCl_6 to an alcoholic solution of the active fraction mentioned above. The activity was associated with this precipitate and was not found in the filtrate. But after decomposition of the precipitate with potassium chloride only a small fraction of the total activity originally present was recovered.

It has been noted that phosphotungstic acid gives a heavy precipitate when added to the aqueous solution of the active alcohol-soluble fraction described above. The decomposition of this precipitate in the ordinary way with hot baryta solution is obviously impossible, but the method used by Van Slyke (4), which consists in suspending the precipitate in acid and shaking out the phosphotungstic acid with a mixture of amyl alcohol and ether, seemed to offer a possible process. It was established that phosphotungstic acid precipitated the oxytocic principle practically completely from acid solution, and by applying Van Slyke's method to the precipitate about half the activity present in the original solution was recovered. Several experiments on the method were made, varying the condition of acidity in the precipitation and decomposition of the precipitate, but in no case could more than approximately half of the activity be recovered. It was observed that there was always a small amount of flocculent material which did not go into solution during the extraction and it may be that the loss of activity was due to adsorption of the active substance on this insoluble precipitate.

The addition of a saturated aqueous solution of sodium picrate to an aqueous solution of the alcohol-soluble active fraction produced an immediate precipitate of a sticky nature which eventually set to a resinous scale on the walls of the containing vessel. On allowing the solution to stand a crop of well-formed wedge-shaped crystals was deposited. This picrate displayed activity of a high order on the isolated uterus, and after two recrystallizations from water the uterine activity remained constant while the melting point rose from 240°C. only to 242°C. The tyrosine, carbohydrate and tryptophane reactions had disappeared and only the Pauly reaction remained. The base gave a crystalline hydrochloride and precipitates with gold chloride and phosphotungstic acid. The ninhydrin reaction was absent. The oxytocic activity was typically that of a pituitary extract, being abolished by treatment in the cold with normal sodium hydroxide. The substance also possessed the characteristic pituitary action on the blood pressure.

Thus, at first sight, it appeared that the picrate of an active base had been isolated. But a few simple calculations made it evident that the substance could not be the active oxytocic principle originally present in the infundibulum. A dose of $\frac{1}{50}$ mgm. of the picrate in a bath of 100 cc. capacity, equivalent to a dilution of 1:5,000,000, produced a maximal contraction of the horn of a guinea-pig's uterus. Assuming thus picrate to be about two-thirds by weight picric acid the effective concentration of the base would be 1:15,000,000. A third of this amount produced a definite, but not nearly maximal, response from the uterus. The substance had, in fact, activity of the same order as that of histamine.

Now, if an extract of *fresh* infundibula is made it is found that 0.5 cc. of a 0.01 per cent (dry matter) extract gives a maximal response on the isolated uterus in the same apparatus and under the same conditions of testing as in the case of the active picrate. That is to say, the extract made from $\frac{1}{20}$ mgm. of dry infundibular matter gives a maximal contraction of the uterus. We know (1) that water extracts about 20 per cent by weight of the dry powder, therefore the actual amount of solid material re-

sponsible for the contraction is approximately $\frac{1}{100}$ mgm. Now a similar maximal contraction was given by $\frac{1}{50}$ mgm. of the active picrate, i.e., by $\frac{1}{100}$ to $\frac{1}{150}$ mgm. of the base. It followed, of course, that if the base isolated above as picrate were indeed the true oxytocic principle of the pituitary the whole of the solid matter extracted from the powder by water must have consisted of the active substance in a practically pure state. And further, this being 20 per cent of the weight of the dry, fat-free infundibular powder, it would mean that about *one-fifth* of the dry matter of the infundibulum consisted of a pure active chemical substance. This would apply, too, incidentally, to histamine, or any substance having a similar order of activity, to which the pituitary uterine activity might be ascribed. Such a supposition appeared to be, if not absurd, at any rate wildly improbable.

It appeared obvious then that the base isolated as picrate could not be the true uterine stimulant of the pituitary gland, but, on account of the apparent constancy of activity and melting point on recrystallization from water, it seemed just possible that it might be a definite chemical compound, a derivative of the actual oxytocic principle, standing in some such relationship to the latter as choline to acetylcholine.

In order to attack the problem of the constitution of the picrate all the remaining butyl alcohol extract was used, and from it about 10 grams of the active alcohol-soluble fraction was obtained. One and three-quarters grams of the picrate was secured, as in the original small scale experiment, in the form of stout wedge-shaped crystals. On searching for a good method of recrystallization it was found that after one recrystallization from 50 per cent alcohol the substance came out as stout, yellow-brown, prismatic needles, and after a second recrystallization as slender long orange-yellow needles. The picrate, thus recrystallized, had only $\frac{1}{400}$ of the activity of the original wedge-shaped crystals on the uterus and only a very slight effect on the blood pressure.

The picrate, therefore, proved to be an inert substance and was merely contaminated with the pituitary active principles. The fortuitous constancy of its activity and melting point on

recrystallization from water were responsible for the temporary assumption that it was an active base. It was successfully identified as the double picrate of creatinine and potassium. No significance is attached to the finding of creatinine in connection with the active principles of the pituitary gland. It is apparently not common knowledge, though it has previously been stated that nervous tissue (brain) is relatively rich in creatinine (5, 6).

The typical pituitary activities on plain muscle and blood pressure were found in the alcoholic mother liquor from the purification of the potassium creatinine picrate, and the active residue therein was fractionally crystallized from increasingly strong alcohol, four small fractions being obtained. The final fraction, a small residue soluble in absolute alcohol, was found to possess the greatest oxytocic activity. It was dried and treated with dry acetone. A good deal went into solution and the very small flocculent precipitate remaining undissolved was filtered off. When dry it appeared as a brownish, non-deliquescent powder. This powder (*A*) proved to be the most active uterine stimulant that has been so far isolated in the course of these experiments. It was about twelve times as active on the isolated uterus as histamine. It produced a good contraction in a concentration of 1:1,250,000,000. The residue (*R*) left after evaporation of the acetone formed a resinous, deliquescent scale. It, too, had a marked activity on the uterus, though only a twentieth of that of *A*.

Interesting results were obtained when the attempt was made to determine the distribution of pressor principle between *A* and *R*. Before this final acetone treatment all the fractions which were active on the uterus displayed the typical pituitary extract activity on the blood pressure, namely, a slight initial depressor effect followed by a powerful and prolonged pressor action. It is well-known that the first dose of a pituitary extract causes a considerably larger pressor response than those which follow, but, after the first few, the rise of blood pressure due to successive equal doses remains fairly constant if the injections are made at equal time intervals. When *A* was tested on the

blood pressure the first dose of 0.01 mgm. gave a marked pressor effect, but a tolerance was quickly developed, so that successive doses produced diminishing responses until, finally, 0.1 mgm. had very little effect. In no case does the injection of *A* give rise to the slightest preliminary depressor action. Now doses of 0.5 mgm. *R* showed a strong depressor effect followed by a moderate pressor response. A slight tolerance was developed to *R*, but strikingly less than in the case of *A*. It was quite impossible to match *A* and *R*, and there can be little doubt that we are dealing here with two distinct substances influencing the blood pressure.

In the previous paper (1) dealing with the extraction of the oxytocic principle by butyl alcohol it was pointed out that at a time when the oxytocic substance is completely extracted only about 50 per cent of the pressor principle has been removed from the aqueous extract. This was taken as definite evidence that the uterine stimulant and pressor substance were distinct chemical individuals.

It was thought desirable to test this observation again on the most active preparation in our possession, since oxytocic and pressor activities had constantly appeared together in all the fractionations attempted in this research. To this end the active fraction (*A*) was dissolved in water and extracted in vacuo with butyl alcohol for ten hours. At the end of this period it was found that about one-half of the oxytocic appeared in the extract, while approximately one-third still remained in the aqueous solution. About one sixth is therefore unaccounted for, suggesting a slight destruction of the substance under the conditions of the experiment. This may be due to the fact that it is less protected by accompanying impurities than in the extraction of the ordinary aqueous extract. On comparison of the butyl alcohol extract and aqueous solution with regard to the distribution of the pressor principle, very little was found in the butyl alcohol and nearly all of it remained still in the aqueous layer. There can, therefore, be no question that the uterine stimulant and pressor principle are two distinct chemical substances.

As only a few milligrams of the fractions *A* and *R* were obtained it was impossible to investigate them chemically except for the sensitive color reactions given by Pauly's and Millon's reagents and the biuret test. The only positive reaction was Pauly's, the color given by *A* being more intense than that given by *R*.

The chemical nature of the active principles of the pituitary gland remains almost completely in the dark. There is really no satisfactory direct evidence even that they are bases. In view of the ease with which they are adsorbed on various precipitates it is not sufficient to state that they appear to be precipitated by platinic chloride, phosphotungstic or picric acids. They may be simply adsorbed on the salts of other bases thrown down in their presence. The fact that the oxytocic principle is destroyed by trypsin and erepsin suggests a peptide-like structure, while its behavior to alkali and acid invites the belief that it may possess an ester linkage. The Pauly reaction, which is consistently present in all active fractions, indicates the possibility that it may be an iminazole derivative.

This investigation shows clearly that the uterine stimulant must be a substance possessing a much higher order of activity than any known at present. The most active preparation obtained, possessing an activity about twelve times that of histamine, was obviously an impure mixture and may well have contained no more than 10 per cent of the actual active principle. The ordinary methods, applied with due regard to the instability of the substance, appear to be inadequate to effect its successful separation. From time to time there have appeared in the literature announcements of the isolation of crystalline derivatives of the pituitary active principles, e.g., picrate (Aldrich) and phosphotungstates (Fühner), but it is highly probable that these have been salts of inactive substances merely contaminated with the active principle as in the case of the potassium creatinine picrate reported in this paper. This experience emphasizes the extreme caution necessary to avoid being misled by such occurrences.

I wish to express my thanks to Miss Florence Durham for much careful help in the numerous tests on the isolated uterus, and I am greatly indebted to Dr. H. H. Dale not only for performing the necessary blood pressure experiments, but also for most helpful advice and criticism throughout the course of the investigation.

ISOLATION OF POTASSIUM CREATININE PICRATE

From about 10 grams of the active alcohol-soluble, ether-precipitated fraction, mentioned in the introduction, a yield of 1.74 grams of the picrate was obtained in the form of stout wedge-shaped crystals. Of this crop 0.0179 gram were set aside for tests (P 1). The remainder, 1.73 grams, was recrystallized from 50 cc. 50 per cent alcohol. It came out of solution in short, stout, yellow-brown prismatic needles. These were filtered off, washed with 8 cc. 50 per cent alcohol and air-dried. Yield 1.265 grams of which 0.0158 gram were set aside for tests (P 2).

The remainder was recrystallized again from 50 cc. 50 per cent alcohol. The crystalline appearance changed, the substance forming very long, slender, golden-yellow needles. Yield (air dried) 1.06 grams (P 3). Simultaneous melting points were taken:

	P 1	P 2	P 3
	°C.	°C.	°C.
Darkened.....	200	210	210
Decomposed, frothing up the tube.....	232	240	242

The point of decomposition varies with the rate of heating. It is absolutely necessary for comparisons to make simultaneous determinations.

The picrates were then tested for activity on the isolated horn of the guinea-pig's uterus.

The following solutions were made for the test:

P 1: 0.01 mgm. per cubic centimeter

P 2: 0.5 mgm. per cubic centimeter

P 3: 1.0 mgm. per cubic centimeter

From the tracing it is apparent that the picrate after two recrystallizations from 50 per cent alcohol has not more than $\frac{1}{400}$ of the activity of the picrate originally crystallizing from water.

The picrates of choline, histidine, and lysine were prepared, and by comparison of properties and mixed melting point determinations were shown to be unlike the picrate isolated from the pituitary preparation. It was noticed that if a crystal of the picrate were dissolved in water and a drop of NaOH added

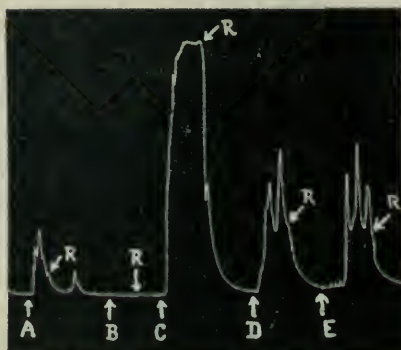


FIG. 1. ACTIVITY OF THE PICRATE ON UTERINE MUSCLE AFTER RECRYSTALLIZATION FROM 50 PER CENT ALCOHOL

At A, 0.5 cc. P 2 solution added.

At B, 1.0 cc. of a sodium picrate containing 1 mgm. per cubic centimeter.

At C, 0.5 cc. P 1 solution.

At D, 1.0 cc. P 3 solution.

At E, 0.25 cc. P 1 solution.

At R, the Ringer solution was changed.

the solution assumed a deep red color. Then 0.498 gram of the picrate was dissolved in 50 cc. of hot water, 10 cc. N HCl were added and the picric acid was removed by shaking out with ether. The solution was taken to dryness in vacuo and a crystalline hydrochloride was obtained composed mainly of needles. Some cubes were also seen. A scrap of this material gave the typical nitroprusside reaction for creatinine, and a further small quantity treated with zinc chloride solution and sodium acetate yielded typical crystals of creatinine zinc chloride. Direct

comparison of the picrate with a specimen of creatinine picrate showed that they were not identical, but a further comparison with the double picrate of creatinine and potassium revealed complete identity (crystalline form, melting point, and behavior on heating in platinum foil), except that the pituitary picrate had a slightly darker color than the specimen prepared from creatinine. This was undoubtedly due to the slight contamination still adhering to the recrystallized picrate from the pituitary material.

Finally, the dry hydrochloride, mentioned above, was treated with hot absolute alcohol and filtered from a small white crystalline residue. This was identified as potassium chloride by the flame test and the preparation of the characteristic insoluble potassium chloroplatinate. The hydrochloride extracted by alcohol gave a faint but definite Pauly reaction.

INVESTIGATION OF THE MOTHER LIQUOR FROM THE CRYSTALLIZATION OF THE POTASSIUM CREATININE PICRATE

The alcoholic mother liquor was evaporated to dryness in vacuo and the residue was taken up in 15 cc. hot 50 per cent alcohol. A small amount of needles separated out. They were filtered off and washed with 50 per cent alcohol. On standing in the cold room for two days spherical nodules were deposited from the solution. These were filtered off, washed and dried. The filtrate was then taken to dryness in vacuo. The residue was dissolved in 20 cc. absolute alcohol. Practically nothing separated from this solution, which was accordingly concentrated in vacuo to 10 cc. when a further deposit of nodules took place. The filtrate from these was taken to dryness in vacuo. All these fractions were tested on the guinea-pig's uterus and all displayed a high order of activity, that of the final alcohol residue being notably higher than the others. This residue was treated with pure dry acetone in the cold. The major part went into solution, leaving a small flocculent precipitate which was filtered off and dried. It was a brownish, non-deliquescent powder, weighing about 20 mgm.

This residue (A) was tested on the isolated guinea-pig's uterus and compared with histamine.

A solution of (*A*) was made containing 0.1 mgm. per cubic centimeter. This was diluted 800 times and used for the test. From the tracing $\frac{1}{4000}$ mgm. (*A*) is more active than $\frac{1}{500}$ mgm. histamine, and $\frac{1}{8000}$ mgm. *A* is less active than $\frac{1}{500}$ mgm. histamine. *A* may therefore be considered to be about twelve times as active as histamine.

On evaporation of the acetone filtrate from (*A*) a resinous scale was left, which was very deliquescent (*R*). This was tested on the isolated uterus against *A*.

A solution of *A* containing 0.1 mgm. per cubic centimeter was made and diluted 100 times; a solution of *R* containing 1.0 mgm. per cubic centimeter was made and diluted 100 times.

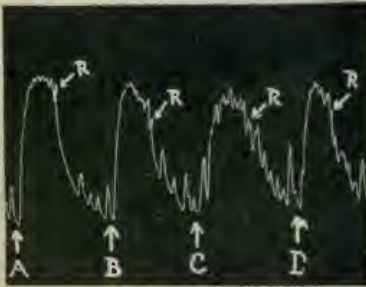


FIG. 2

FIG. 2. COMPARISON OF FRACTION *A* WITH HISTAMINE (OXYTOMIC ACTIVITY)

At A, 2 cc. *A* solution was added.

At B, a solution containing 0.002 mgm. histamine was added.

At C, 1 cc. *A* solution was added.

At D, a solution containing 0.002 mgm. histamine was added.

At R, change of Ringer solution.

Volume of bath 125 cc.



FIG. 3

FIG. 3. COMPARISON OF UTERINE ACTIVITIES OF *A* AND *R*

At A, 0.1 cc. solution *A* was added.

At B, 0.3 cc. solution *A* was added.

At C, 0.6 cc. solution *R* was added.

At R, Ringer solution was changed.

Volume of bath 125 cc.

It will be noted from the tracing that *A* is twenty times as active as *R*, and also that *A* produced a definite response in a dose of $\frac{1}{10,000}$ mgm., that is to say in a concentration of 1:1,250,000,000.

THE EFFECT OF (*A*) AND (*R*) ON THE BLOOD PRESSURE

The following points should be noted:

The strong, *pure* pressor effect produced by the first dose of 0.01 mgm. *A* at *A* 1.

The rapidly developing tolerance to *A*, shown by the responses at *A* 2 and *A* 3.

The noticeable depressor effect at the first small injection of *R* at *R* 1.

The tolerance developed to *A* as shown at *A* 4, *A* 5, and *A* 6 compared with that for *R* as shown at *R* 2, *R* 3, *R* 4 and *R* 5.

The entire absence of any trace of depressor effect following injections of *A* throughout the experiment.

DIFFERENTIATION BETWEEN OXYTOCIC AND PRESSOR PRINCIPLES OF (*A*) BY MEANS OF BUTYL ALCOHOL EXTRACTION

The total remaining quantity of *A* (0.0138 gram) was dissolved in 41 c.c. water giving a solution of 1 mgm. in 3 c.c. Five cubic centimeters of this solution were sterilized and put aside for tests. The remainder was extracted with butyl alcohol continuously under reduced pressure for ten hours. The residues from the aqueous layer (*P*) and from the butyl alcohol (*H*) were dissolved each in 36 cc. water, i.e., the original volume of the extracted aqueous solution. A portion of the 5 cc. set aside was diluted to give a solution containing 0.001 mgm. per centimeter cubic *A*. Five cubic centimeters of each of the solutions *P* and *H* were diluted to 8.3 cc. giving solutions of a nominal strength of 0.2 mgm. per cubic centimeter. These solutions were diluted 100 times and then used for the test for oxytocic activity.

From the tracing $0.8 H$ (diluted) = $0.7 A$ and $0.9 P$ (diluted) = $0.6 A$ whence $\frac{7}{16}$ of the uterine stimulant have been extracted by butyl alcohol and $\frac{1}{3}$ remains in the aqueous solution, leaving a little less than $\frac{1}{4}$ unaccounted for.

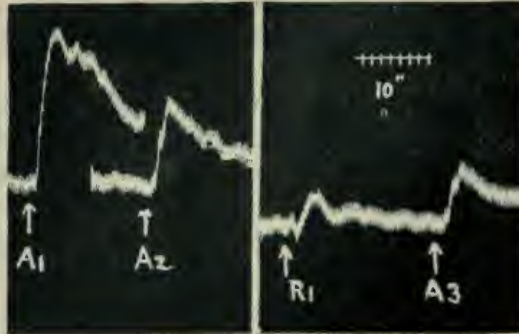


FIG. 4

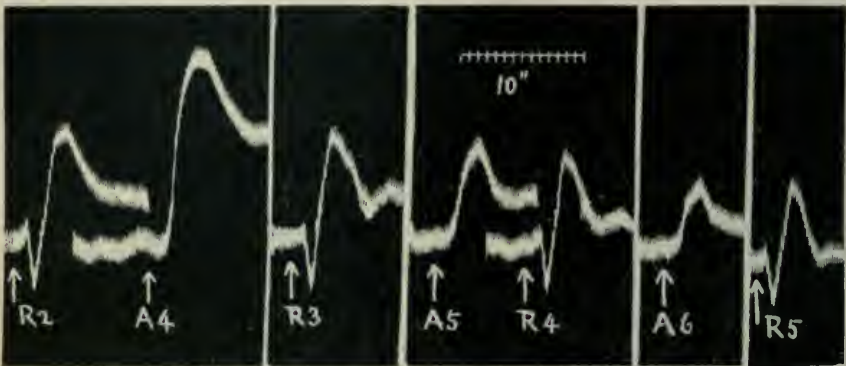


FIG. 5

FIGS. 4 AND 5. EFFECT OF A AND R ON THE BLOOD PRESSURE

Decerebrated cat, 3.5 kilos. (Both figures 4 and 5 are parts of the same tracing.)

At A 1 the first injection of the experiment was made, 0.01 mgm. *A* being introduced. Six minutes later at A 2 another injection of 0.01 mgm. *A* was made.

At R 1, twenty minutes after A 2, 0.1 mgm. *R* was injected. At A 3, three minutes after R 1, 0.01 mgm. *A* was injected.

During the next one and one-half hours two injections of *A* and two of *R* were made in an unsuccessful attempt to find a match. After an interval of fifteen minutes, following a dose of 0.02 mgm. *A*, the series (shown in fig. 5) commencing at R 2 was obtained.

AT	DOSE	TIME INTERVAL BETWEEN DOSES
R 2	0.5 mgm. (<i>R</i>)	Two hours after first injection at A 1
A 4	0.1 mgm. (<i>A</i>)	
R 3	0.5 mgm. (<i>R</i>)	23 minutes
A 5	0.1 mgm. (<i>A</i>)	7 minutes
R 4	0.5 mgm. (<i>R</i>)	7 minutes
A 6	0.1 mgm. (<i>A</i>)	7 minutes
R 5	0.5 mgm. (<i>R</i>)	7 minutes

The distribution of pressor substance was now determined.

The injection at A was the first of the experiment and is, therefore, an exaggerated response to the dose. In spite of this it is very small. The injection at B is, for practical purposes,



FIG. 6. EXTRACTION OF A BY BUTYL ALCOHOL; DISTRIBUTION OF OXYTIC PRINCIPLE

At A, 0.8 cc. *H* (diluted) was added.

At B, 0.7 cc. *A* was added.

At C, 0.9 cc. *P* (diluted) was added.

At D, 0.6 cc. *A* was added.

At R, Ringer solution was changed.

Volume of bath 125 cc.

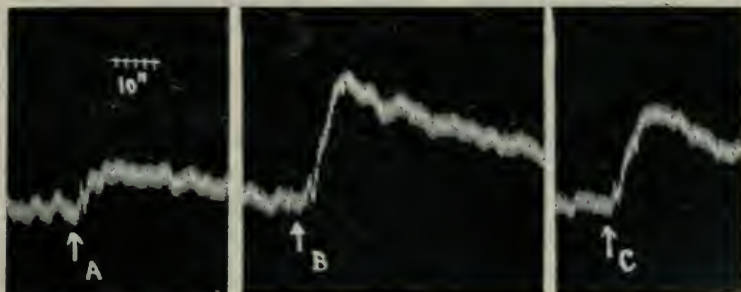


FIG 7. EXTRACTION OF A BY BUTYL ALCOHOL; DISTRIBUTION OF PRESSOR PRINCIPLE

Decerebrated cat

At A, injected a solution containing nominally 0.01 mgm. *H* at 11.25 a.m.

At B, injected a solution containing nominally 0.01 mgm. *P* at 11.30 a.m.

At C, injected a solution containing nominally 0.01 mgm. *A* at 11.45 a.m.

the first vigorous response to the pressor principle, and is, doubtless, somewhat greater than would be given by a subsequent dose. It is obvious that very little indeed of the pressor principle has been extracted from the aqueous layer, while about half of the oxytocic substance has been removed. It is, therefore, certain that the oxytocic and pressor substances in question are two separate chemical individuals.

A NOTE ON THE RECENT PAPER OF ABEL AND ROUILLER

Since this paper was written a communication by Abel and Rouiller has appeared (7). These authors, by a procedure differing at many points from mine, have obtained a preparation of the pituitary principles with an activity of the same order as that of my most active fraction. They estimate the oxytocic activity of their preparation as twenty to thirty times that of histamine phosphate; my most active fraction was found to be about 12 times as powerful a uterine stimulant as histamine base, i.e., about thirty-three times as active as histamine diphosphate. They make no claim that their preparation is a pure substance expressing a confident belief "that the entirely uninjured principle will be found to be at least forty to fifty times more powerful than histamine in its action on the uterus of the virgin guinea-pig." With this conclusion, namely, that the oxytocic pituitary principle has an activity very many times as powerful as that of histamine and greater than that of any known chemical substance, I am in full agreement.

Abel and Rouiller's other conclusion, that "the vasomotor, oxytocic and renal activity of (their) preparations are only the expression of the manifold physiological properties of one and the same hormone" I am unable to accept. With the effect on the function of the kidney I am not directly concerned, as I have made no experiments on it with my own preparations. The action which Abel and Rouiller mention is the diminution or entire inhibition of a previously active urinary flow in the rabbit; and it is only necessary to point out that a proof of the identity of the principle producing this effect with the pressor principle, if obtained, would hardly justify a similar assumption concern-

ing the principle causing the stimulation of urinary secretion, which is the effect on the kidney described by Schafer and his co-workers.

The rest of the statement, read in connection with the body of their paper, is found to involve the attribution to the principle of three other effects, namely, first, the oxytotic action, second, the pressor effect of first injections, and third, the depressor effect often caused by later injections of the same preparation. The evidence in favor of this identity is the close and persistent association of these different kinds of action through various manipulations and their similar sensitiveness to destructive agencies, such as alkali or tryptic digestion. These correspondences are familiar to every worker who has attempted the purification of the active principles, and they are, indeed, sufficiently numerous to suggest strongly that the different actions may be due to principles having a fundamental similarity of constitution. Nothing, however, short of the isolation of one pure substance, having all these different physiological properties, could be accepted as proof of *identity*.

On the other hand, no absolute or even approximate purification is necessary for the disproof of identity; if by any methods the different types of activity can be separated into different fractions even of impure material, we have evidence of the multiplicity of the active principles against which no evidence of failure to separate, by other methods of fractionation or destruction, has any weight.

In 1919, I described a method by which a partial separation of the oxytotic and pressor principles could be effected. It consisted in the extraction of the aqueous solution with butyl alcohol. Abel and Rouiller have apparently overlooked this evidence, which is fatal to their assumption of identity. The procedure is a simple one which could have been applied easily to their "nearly pure" solution of active substance. I have shown in the foregoing paper that butyl alcohol extraction of a preparation of the same order of activity as theirs removes the oxytotic principle at a much greater rate than the pressor, which remains for the most part behind in the aqueous layer.

Abel and Rouiller believe that their evidence further points to the production of depressor effects, with later injections, by the same principle which produces an apparently pure pressor effect with the first. Here again their only evidence is the persistence of both these effects in their "nearly pure" solution, and such evidence can have no weight against my evidence of the separation of the two effects in the experiment in which the active residue, obtained from the potassium creatinine picrate mother liquor, was fractionated by means of acetone. The fraction *A* insoluble in acetone, which incidentally was the most powerful oxytocic substance obtained, had a pure pressor action in all experiments, not only with the first, but with all subsequent doses. An initial dose of 0.01 mgm. had a pronounced pressor effect in a cat weighing 3.5 kilos (Compare Abel and Rouiller's statement of a similar effect with 0.5 mgm. of their preparation in a dog of 6.4 kilos). Tolerance to *A* was rapidly developed, however, with successive injections, so that eventually even 0.1 mgm. produced only a small pressor effect, though always uncomplicated by any trace of depressor action. The fraction (*R*), soluble in acetone, on the other hand, showed from the first injection a diphasic action—preliminary depressor followed by a pressor effect. With successive doses the pressor effect showed some decline, but the tolerance was not comparable with that established for *A*. In an experiment in which the two preparations were alternated, with the object of determining an equivalence, the pressor effect of 0.1 mgm. *R* was initially very much less than that of 0.01 mgm. *A*; finally 0.5 mgm. *R* had a larger pressor effect than 0.1 mgm. *A*. It was impossible to fix equivalent doses, and the widely different rates at which tolerance was developed seemed to justify the conclusion that even the *pressor effects* of *A* and *R* were due to different principles, quite apart from the question as to whether the depressor and pressor effects exhibited by *R* were due to one or to more than one substance. In either case the depressor effect of *R* was clearly due to a substance not identical with that causing the purely pressor effect of *A*; so that the original mixture from which *A* and *R* were separated and which gave exactly the sequence of blood pressure

effects described by Abel and Rouiller, contained at least two, and probably more than two, active principles. Since *A* was the preparation subsequently separated, by butyl alcohol extraction, into a powerful pressor but weakly oxytocic, and a powerfully oxytocic but weakly pressor fraction we have clear evidence of a further principle, acting powerfully on uterine muscle, with little, if any, pressor action and no depressor action.

So far from it being a nearly pure preparation of a single active principle, producing all the essential physiological effects, I am driven, therefore, to the conclusion that Abel and Rouiller's preparation, like my own, contained at least three, and probably more, active substances mixed with an unknown quantity of inert material.

My conclusion, I am aware, shows the prospect of isolating the pituitary active principles in a far less encouraging light than theirs; but the experience of the past few years has given me so strong an impression of the difficulties entailed in the search for this group of extremely active, unstable substances, present in relatively minute proportions in the scarce and costly material of the posterior lobe of the pituitary gland, that I feel it a duty to make clear, for the benefit of other workers on the subject, my own reading of the facts at present available.

SUMMARY

1. An attempt has been made by various methods to effect a purification of the oxytocic principle of the posterior lobe of the pituitary gland.

2. A crystalline picrate was obtained which had the characteristic actions of pituitary extract on plain muscle and blood pressure. It had an activity of the same order as that of histamine. It was found to be the picrate of an inert substance merely contaminated with small amounts of the pituitary principles, and was eventually identified as potassium creatinine picrate.

3. Out of the mother liquor from the recrystallization of this potassium creatinine picrate a small alcohol soluble residue was obtained. This yielded an acetone insoluble *A* and an acetone soluble *R* fraction. *A* had an oxytocic activity equal to about twelve

times that of histamine (reckoned as base), producing a definite contraction of the uterus in a dilution of 1 part in 1,250,000,000. *A* also contained a pressor principle, displaying no preliminary depressor activity. It produced a strong rise of blood pressure in a dose of 0.01 mgm. *R*, had one-twentieth the oxytocic activity of *A*, and in doses of 0.5 mgm. produced a strong depressor action followed by a moderate pressor action on the blood pressure.

4. Evidence is put forward to show that the oxytocic and pressor principles of *A* are two separate chemical individuals, and that the pressor principles of *A* and *R* are most probably also two distinct chemical entities.

5. It is considered, therefore, that there is valid evidence for the presence of at least three different physiologically active principles in pituitary extracts. Since this view is in direct conflict with that of Abel and Rouiller a criticism of their recently published conclusions is presented.

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COMPARATIVE TOXICITY OF INORGANIC LEAD COMPOUNDS AND METALLIC LEAD FOR PIGEONS

P. J. HANZLIK AND ELIZABETH PRESNO

From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco

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The present study is a continuation of the study of chronic lead poisoning from metallic lead in pigeons previously reported (1). Of all the inorganic lead compounds, the carbonate and acetate have been most extensively studied. The toxicity of the chloride, iodide and sulphide have been scarcely studied at all, although a knowledge of this is desirable, since some of the compounds (chloride and sulphide) are formed in the alimentary tract during lead poisoning and others may be formed during the administration of certain therapeutic agents (sodium and potassium iodide and calcium sulphide). The administration of sodium or potassium iodide in chronic lead poisoning is alleged by some clinicians to mobilize the lead of the tissues and aggravate the symptoms of toxicity. Aside from interest which attaches to these compounds in the development and treatment of lead poisoning, they also constitute sources of chronic poisoning, since they are extensively used in the arts and industry.

METHOD

Adult pigeons of about 350 grams body weight were used in the same way as those poisoned by metallic lead and described in a previous paper (1). The details need not be repeated here. Loss in body weight was the most accurate index of poisoning. Briefly, the pigeons and their food were weighed regularly, and symptoms were recorded as they developed. Two sets of experiments were made. In one set, single doses of lead compounds whose lead content was equivalent to the higher and maximal

fatal dosage of metallic lead (previously reported) were administered into the crop and the pigeons were placed on normal food and observed until the body weight recovered to the previous level. In the other set, continued or daily doses of the compounds were administered by incorporating the compounds with the food. The dosage used corresponded to the higher and maximal fatal doses of metallic lead, as indicated by the quantity of liberated lead (gram per kilo per diem) previously reported. The following compounds were studied; lead chloride, lead iodide, lead acetate, lead sulphide and lead carbonate. Lead sulphide and lead iodide were prepared freshly in the laboratory and the others were pure, analyzed salts obtained in the market. The dosage and the other pertinent data are presented in the accompanying tables. At least three pigeons were used with each compound in each set of experiments except in the case of lead acetate administered in single dosage, in which two pigeons were used. First, the results with single doses of the compounds will be described.

SINGLE DOSES

The results obtained are presented in table 1. It is seen that all the pigeons receiving the different compounds were poisoned though variably as indicated by the marked variations in maximal losses of body weight (range 1.8 to 36 per cent). None of the pigeons died, despite the rather large doses administered, which in terms of metallic lead (0.92 to 2.48 grams per kilo) corresponded to the fatal (0.6 to 2.28 grams per kilo) dosage of lead for the majority (80 to 100 per cent) of pigeons in a previous study (1) receiving lead in the form of shot (metal). This is probably due to deficient retention of the salts as compared with metallic lead when administered in the form of shot.

Quantitatively, lead chloride appeared to be the most toxic of all the compounds tried; next in order was lead acetate, and least toxic were the iodide, carbonate and sulphide. This is indicated by the medians and ranges of maximal percentage loss of body weight, which was most marked with lead chloride (1.8 to 36 per cent); somewhat less (11.2 and 11.9 per cent) with the acetate

TABLE 1
Effects of the administration of single doses of lead compounds to pigeons*

LEAD COMPOUND USED	NUMBER OF PIGEONS USED	BODY WEIGHT OF PIGEONS	DOSEAGE OF LEAD COMPOUND PER KILO ADMINISTERED	EQUIVALENT DOSEAGE OF METALLIC LEAD PER KILO ADMINISTERED	MORTALITY	MAXIMAL LOSS OF BODY WEIGHT	TIME OF REAPPEARANCE OF MAXIMAL LOSS OF BODY WEIGHT	FIRST APPEARANCE OF LOSS OF BODY WEIGHT	TIME OF RECOVERY†	MEDIAN DAILY FOOD CONSUMPTION
		grams	grams	grams		days	days	days	days	grams
Lead chloride.....	3	322 287 300	1.09 1.74 3.3	0.79 1.30 2.48	None None None	1.8 29.3 36.0	6 53 28	4 4 4	13 65+ 28+	22 8 16
Lead iodide.....	3	312 319 338	1.88 3.48 6.00	0.85 1.57 2.70	None None None	10.0 3.4 7.7	4 4 15	4 4 6	16 13 21	23 19 14
Lead carbonate.....	3	372 314 290	0.463 1.18 2.6	0.36 0.92 2.02	None None None	3.0 4.8 7.3	9 9 22	1 2 6	40 23 26	70 16 58
Lead sulphide.....	3	349 317 325	0.82 1.73 2.46	0.71 1.42 2.02	None None None	2.6 4.7 14.1	20 17 27	2 12 6	11 20 27	17 16 18
Lead acetate.....	2	338 270	0.77 1.88	0.42 1.03	None None	11.2 11.9	32 6	4 6	50 27	21 17

* The percentage lead content of the different compounds is as follows; lead chloride, 74.7, lead carbonate, 77.5, lead sulphide, 86.6, lead acetate, 54.6 and lead iodide, 44.9. Accordingly, in equal dosage and providing all other factors are constant, the sulphide would be the most and the iodide the least toxic. Solubility, of course, will determine absorption and, therefore, toxicity to a considerable extent.

† As indicated by return of body weight to previous level.

and least marked with the iodide and carbonate and also sulphide (1 to 14 per cent).

The first appearance of loss of body weight varied somewhat, but was more uniform (average of 4 days after administration) than the time of maximal appearance, which was much more delayed with lead chloride (at end of 6 to 53 days) and lead sulphide (17 to 27 days). These compounds tended to produce a greater percentage loss of body weight than the rest of the compounds studied. The time of appearance of the greatest loss of body weight with the iodide, carbonate and acetate was earlier, namely, at the end of from 4 to 32 days (median, 8 days) after administration of the compounds.

The time of recovery from the effects of the different compounds was judged by return of the pigeons to their previous body weight. It is seen that this was more uniform than the other factors, amounting to a median of 26 days with a range of from 13 to 65 days for all compounds. As compared with pigeons poisoned by metallic lead, recovery of pigeons receiving the various lead compounds took place sooner.

The daily food consumption was only moderately reduced. The median consumption for all pigeons was 18 grams, the range being 8 to 70 grams, as compared with a median of 23 grams, per diem for normal pigeons, and 6 grams for pigeons poisoned by smaller doses of metallic lead. The greatest variation in food consumption occurred in the group of pigeons receiving lead chloride.

On the whole, the results indicate that single large doses of the the lead compounds that were studied cause a temporary and moderate degree of toxicity in pigeons. This was different with metallic lead which was found to be more toxic and caused more permanent injury. Results similar to those with single doses were obtained with continuous, small and large doses of the lead compounds to be described presently.

CONTINUOUS DOSES

The results are summarized in table 2. It is seen that only two lead compounds out of the five that were used caused fatali-

TABLE 2
Effects of the administration of lead compounds in continuous or daily doses with food to pigeons

LEAD COMPOUND USED	NUMBER OF PIGEONS USED	BODY WEIGHT OF PIGEONS	DOSAGE OF LEAD COMPOUND PER KILO PER DIEM ADMINISTERED	EQUIVALENT DOSAGE OF METALLIC LEAD PER KILO PER DIEM ADMINISTERED	NUMBER OF DAYS OBSERVED	MORTALITY	MAXIMAL LOSS OF BODY WEIGHT	TIME OF APPEARANCE OF MAXIMAL LOSS OF BODY WEIGHT	FIRST APPEARANCE OF LOSS OF BODY WEIGHT	MEDIAN DAILY FOOD CONSUMPTION	TIME OF RECOVERY
Lead iodide.....	3	408	0.0304	0.014	128	None	14.4	102	4	18	Incomplete
		329	0.0335	0.015	42	None	6.4	28	7	16	28
		299	0.0643	0.0289	36	Died end of 36 days	38.0	36	3	14	None
Lead carbonate.....	3	330	0.0373	0.029	14	None	0.6	10	7	15	14
		336	0.0220	0.017	38	None	4.7	9	4	18	26
		292	0.1235	0.096	20	None	9.0	17	3	22	20
Lead acetate.....	3	375	0.056	0.031	14	None	0.8	10	7	18	14
		375	0.027	0.015	37	None	4.3	12	4	18	33
		370	0.076	0.042	41	None	4.0	41	4	25	41+
Lead chloride.....	3	383	0.013	0.0097	107	Died end of 190 days	49.6	190	4	12	None
		374	0.020	0.015	41	None	2.0	41	3	18	20
		305	0.0484	0.0363	20	None	4.6	6	6	18	10
Lead sulphide.....	3	410	0.0214	0.019	93	None	9.0	72	4	24	93+
		378	0.0223	0.0193	71	None	9.52	30	4	23	67
		304	0.0265	0.023	64	None	8.2	30	4	22	64

ties, namely, lead iodide and lead chloride. The death of the chloride pigeon may have been due to causes other than lead, since this bird received the smallest dose of chloride and died only at the end of 190 days, which may have represented the end of its natural span of life. Its median daily food consumption was 12 grams, which compares favorably with the daily food consumption (16 grams) of pigeons previously reported and which recovered from a median concentration of metallic lead greater (0.16 gram) per kilo per diem than was administered to this bird. This leaves the single iodide pigeon out of the entire lot of 15 pigeons whose death may be attributed to a lead compound. Even so, the daily dosage per kilo (0.0289 gram) in terms of metallic lead for this iodide pigeon was greater than a somewhat lower daily dosage (0.0214 gram) of metallic lead that can be tolerated by the majority (85 per cent) of pigeons. The remaining iodide pigeons received smaller daily doses, which, however, were somewhat higher than the minimal daily quantity (grams per kilo) liberated in pigeons receiving metallic lead.

The following compounds caused no deaths; lead carbonate, lead acetate and lead sulphide. The range of highest daily dosage per kilo of these compounds used and expressed in terms of metallic lead was greater (0.03 to 0.096 gram) than that of lead liberated from the administration of metallic lead which was fatal to the majority (80 to 100 per cent) of pigeons in a previous study, namely, a range of medians from 0.0214 to 0.1 gram per kilo per diem.

As far as maximal loss of body weight is concerned, the two pigeons, which died (receiving iodide and chloride) showed losses corresponding to those which received metallic lead, namely, 38 and 49.6 per cent, respectively. The remaining pigeons showed only small (2 to 14.4 per cent) and temporary losses of body weight, recovery to their previous weights occurring in from 10 to 93 days.

The first appearance of loss of body weight in all pigeons was demonstrable somewhat later, namely, in from 3 to 7 days after administration of the lead, than with the pigeons receiving metallic lead in a previous study (in from 2 to 4 days). This

indicates that the lead compounds act more slowly, although theoretically the absorption of lead compounds should be quite as good as that of metallic lead.

The time of appearance of the maximal loss of body weight varied markedly with all pigeons and also among individual groups receiving the different compounds. The range for all pigeons was from 6 to 190 days, while that for all pigeons receiving different doses of metallic lead alone was 3 to 83 days with a median value of 21 days for those that survived and 17 days for those fatally poisoned. This again indicates that the toxic action of lead compounds is slower and weaker than that of metallic lead administered to pigeons under similar conditions. An analysis of the difference of the behavior of lead in these two forms (metal and salts) was not attempted.

As compared with the median daily food consumption of normal pigeons (23 grams) previously reported, the median daily food consumption of all the pigeons receiving continuous doses of different lead compounds was moderately reduced, constituting a range of from 12 to 24 grams. The individual median daily food consumption was generally higher than that (16 grams) of pigeons receiving non-fatal doses of metallic lead. This is in line with the less pronounced effects on body weight and low mortality described above, all of which gives the impression that the various lead compounds which were used in this study are less toxic than metallic lead for pigeons under similar conditions of experimentation.

CONCLUSION

The following lead compounds; chloride, iodide, sulphide, carbonate and acetate, administered in single, and continuous or daily doses, whose lead content was equivalent to and greater than the fatal dosage of metallic lead, were less toxic than metallic lead for pigeons under similar conditions of experimentation.

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THERAPEUTIC EFFICIENCY OF VARIOUS AGENTS FOR CHRONIC POISONING BY METALLIC LEAD IN PIGEONS

P. J. HANZLIK AND ELIZABETH PRESNO

*From the Department of Pharmacology, School of Medicine, Stanford University,
San Francisco*

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Among the most commonly used agents in the treatment of chronic lead poisoning are iodide (sodium or potassium), magnesium sulphate and calcium sulphide. Clinically their efficacy is conjectural. This is due to a lack of appreciation that spontaneous recovery can occur without medication and the difficulty of obtaining satisfactory controls. The rationale of using the iodide and sulphide is obscure. For instance, iodide is used by some with the idea of facilitating the excretion (through salt action) of lead, and by others with the idea of mobilizing the metal which is assumed to be stored in the tissues. In large and continued doses it is alleged by some to be detrimental, aggravating the symptoms of poisoning by excessive mobilization of lead. Calcium sulphide is assumed to act by reducing the solubility of the lead in the tissues and hence its toxicity. None of these hypotheses is supported by experimental or clinical data. The beneficial effects of magnesium sulphate are attributed, more rationally, to its cathartic action.

The experimental chronic lead poisoning of pigeons previously described (1) offers an opportunity for testing therapeutic agents under controlled conditions. It is the object of this paper to present the results that were obtained with iodide, magnesium sulphate and calcium sulphide administered to pigeons poisoned by metallic lead. Briefly, the results obtained are favorable to the clinical usage of these agents. However, the results are not transferable to the treatment of chronic lead poisoning in

man under all conditions for the following reasons: First, poisoning in man may be due to compounds of lead instead of metallic lead, and from channels other than the alimentary tract; second, therapeutic agents are commonly used in the absence of exposure to lead, whereas the pigeons in this study received the therapeutic agents while they were continuously poisoned by metallic lead which was ground and liberated in the gizzard. Nevertheless, it is believed that the special conditions (more or less) prevailing in the pigeon experiments give a value to the results in other directions. They are applicable to those cases of poisoning harboring unabsorbed lead; under factory conditions, and when these agents are used as prophylactics. The use of metallic lead in the experiments is not objectionable because eventually the lead, whether derived from shot or compounds, is acted upon by the digestive juices and rendered soluble and absorbable. In fact, in a previous study (2) metallic lead was found to be more toxic than various lead compounds for pigeons. Therefore, the use of metallic lead in pigeons imposes more severe conditions, if anything, for testing therapeutic agents. Finally, the results give us some idea for the first time of the efficacy of the therapeutic agents that were tested under controlled conditions.

METHODS

Metallic lead in the form of shot (no. 6) was administered to adult pigeons of about 350 grams body weight in the same way as was described in the first paper (1) of this series. The details pertaining to weighing of pigeons and determining their food consumption will be omitted in this paper. The dosage of metallic lead corresponded to the upper limits of fatal dosage for the majority of pigeons. The therapeutic agents were mixed with the food in concentrations, which provided for a daily dosage corresponding to the maximal therapeutic dosage for man. Recovery was judged by the return of body weight to the previous level. The effects of iodide were tested on 10 pigeons, and those of magnesium sulphate and calcium sulphide

on 3 each. Sodium chloride was administered as a control to 3 pigeons. The dosage of lead and therapeutic agents together with other data are presented in the accompanying table.

RESULTS

Sodium chloride. This was used as a control for the iodide and calcium sulphide. It is seen from the results in the accompanying table that the 3 leaded pigeons which received daily doses of sodium chloride ranging from 0.0245 to 0.0378 gram died. The dosage of lead used (0.8 to 1.12 grams per kilo) corresponded to that (0.6 to 2.28 grams) per kilo which was found to be fatal for the majority (80 to 100 per cent) of pigeons in a previous study (1). The remaining criteria (using median values) of poisoning also had the same tendencies quantitatively, namely, time of death occurred at end of 18 days, maximal loss of body weight was 22.3 per cent, first appearance of loss of body weight occurred at end of 4 days and the time of maximal loss at end of 18 days; daily food consumption was 11 grams, as compared with 20 days, 35 per cent, $3\frac{1}{2}$ days, 16 days and 4.5 grams, respectively, for pigeons receiving fatal doses of lead alone. This was different with the therapeutic agents that were tested.

Iodide. Two sets of experiments were made with sodium iodide. In one set, 8 pigeons received iodide in concentrations from 0.11 to 0.44 per cent in food, and in the other set, 5 pigeons received a concentration of 0.11 per cent iodide in drinking water, the food remaining untreated. A concentration of 0.11 per cent of iodide for a pigeon of 350 grams with a normal daily food consumption of 23 grams is equivalent to a daily dosage of about 4.3 grams of sodium iodide for an adult man of 60 kilos; a 0.22 per cent concentration would be the equivalent of 8.6 grams, and 0.44 per cent, 17.3 grams. The iodide was administered on the assumption that the pigeons would maintain a nearly normal daily food consumption of 23 grams (previously reported), and actually the consumption was close to this with a majority of the pigeons, that is, about 20 grams. It was only markedly reduced (to 0.7 gram) in those pigeons which died

TABLE 1
Effect of various therapeutic agents on poisoning by metallic lead in pigeons

NUMBER OF PIGEONS USED	BODY WEIGHT	DOSAGE OF LEAD ADMIN- ISTERED	DAILY DOSAGE OF THERAPEUTIC AGENT PER KILO	MORTALITY	TIME OF RECOVERY IN SURVIVAL CASES	TIME OF DEATH IN FATAL CASES	MAXIMAL LOSS OF BODY WEIGHT	TIME OF APPEAR- ANCE OF MAXIMAL LOSS OF BODY WEIGHT	FIRST AP- PEARANCE OF LOSS OF BODY WEIGHT	MEDIAN DAILY FOOD CONSUM- TION		
0.11 per cent NaCl in food												
3	grams	grams per kilo		per cent	days	days	per cent	days	days	grams		
	{	291	0.9	0.04	gram for 18 days	18	22.3	18	4	10		
		449	1.12	0.0245	gram for 18 days	18	10.0	11	4	10		
	321	0.8	0.0378	gram for 27 days	27	34.6	21	4	9.6			
0.11 per cent, 0.22 per cent and 0.44 per cent iodide in food												
8	{	379	0.69	0.058	gram for 116 days	113 66	10.0	83	2	20		
		358	0.76	0.056	gram for 77 days			23	2	18		
		301	1.66	0.004	gram for 14 days*			14	46.5	14	4	0.7
		237	2.10	0.093	gram for 15 days*			16	38.4	16	4	20
		385	0.67	0.0571	gram for 148 days	148 (incomplete) 86	35.3	16	2	20		
		276	0.98	0.0566	gram for 109 days		28.6	16	2	14.2		
		338	1.18	0.0456	gram for 33 days*†	33	38.4	33	1	7		
		388	0.77	0.228	gram for 23 days†	22	7.7	8	1	20		
0.11 per cent iodide in water												
5	{	259	1.46	0.0552	gram for 100 days	97	14.0	28	4	14		
		364	1.05	0.078	gram for 30 days		5.2	7	4	64		
		280	1.63	0.0670	gram for 15 days*	20	15	39.6	15	4	3	
		246	1.97	0.0626	gram for 64 days		40	39.0	15	8	16	
		445	0.92	0.0830	gram for 9 days		7	4.6	2	2	33	

0.66 per cent magnesium sulfate in food

3	{	339 347 388	gram for			None	70 23 57	32.0 18.4 23.0	37 8 23	2 1 2	20 16 22	
			0.74 1.15 1.30	71 days								0.39 0.304 0.374
				gram for	23 days							
				57 days								

0.22 per cent calcium sulfide in food

3	{	326 314 377	gram for 31 days			None	22 47 47	3.7 1.5 3.5	9 25 18	3 4 4	15 27 21	
			1.13 1.12 0.94	0.10 0.19 0.122								gram for 50 days

* Died.

† Administered as 0.22 per cent sodium iodide.

‡ Administered as 0.44 per cent sodium iodide. Remaining pigeons received 0.11 per cent iodide in food.

while receiving iodide in food, but was greatly augmented in surviving birds receiving iodide in water (up to 64 grams, median).

The results show that concentrations of iodide in food ranging from 0.11 to 0.44 per cent, or a median daily dosage of 0.058 gram per kilo, were beneficial for poisoning by doses of lead ranging from 0.69 to 0.98 gram per kilo. Out of the 5 pigeons used with this dosage none died, while the mortality for a corresponding range of dosage (0.6 to 0.86 gram per kilo) of metallic lead alone was $83\frac{1}{2}$ per cent. The remaining criteria (using median values) also showed beneficial tendencies, namely, maximal loss of body weight was 21 per cent, first appearance of loss of body weight was 2 days and time of appearance of maximal loss was 16 days, daily food consumption was 20 grams as compared with 40 per cent, 4 days, 24 days and 3 grams, respectively, for untreated pigeons receiving a similar dosage of lead alone.

Daily doses of sodium iodide ranging from 0.004 to 0.093 grams per kilo (median, 0.05 gram daily) did not markedly alter the toxicity of higher doses of metallic lead, namely, 1.18 to 2.1 grams, per kilo, which corresponded to the highest dosage of lead in untreated pigeons previously reported. This is indicated by the unfavorable tendency of all criteria (median values) used for judging the therapeutic effects. The mortality was 100 per cent, maximal loss of body weight 38.4 per cent, and this appeared at end of 16 days after administration of the lead and the daily food consumption was only 7 grams. The figures for the same criteria with the same dosage of metallic lead in untreated pigeons were practically the same and need not be repeated. This indicates that iodide is therapeutically beneficial for moderately high fatal, but not the highest fatal doses of metallic lead. Higher doses of sodium iodide in a larger series of pigeons might give better results.

On the other hand, sodium iodide in drinking water was more efficient against the higher doses of lead. Out of 5 pigeons that received a range of doses from 0.92 to 1.97 grams per kilo of lead only 1 died (mortality, 20 per cent). The dosage of lead used practically corresponded to the highest range of

doses (0.95 to 2.28 grams per kilo) of lead in untreated pigeons in whom the mortality ranged from 80 to 100 per cent. The daily dosage of iodide ranged from 0.055 to 0.083 gram per kilo, which is nearly the same as the dosage of iodide in food. Apparently, dissolved iodide (in drinking water) is more efficient. The remaining criteria were variably though, in general, beneficially influenced. The maximal loss of body weight was only 4.6 and 5.2 per cent, with doses of 0.92 and 1.05 gram per kilo, and considerably higher (median, 39 per cent) with doses of lead ranging from 1.46 to 1.97 gram per kilo. The first appearance of loss of body weight was at the end of 2 and 4 days with the two lower doses and a median of 6 days with the three higher doses of lead. The maximal loss of body weight appeared at the end of 2 and 7 days with the lower doses and a median of 15 days with the higher doses. The values for the criteria in untreated leaded pigeons receiving doses of metallic lead corresponding to the higher doses (1.46 to 1.97 grams per kilo) were practically the same, indicating that iodide did not greatly alter the toxicity, although the mortality was reduced. The values for the smaller range of dosage (0.92 to 1.05 gram per kilo) treated by iodide in water had a more beneficial tendency than for a corresponding range of dosage in untreated pigeons.

The food consumption was greatly increased by all pigeons treated with iodide in water. This amounted to daily consumptions of 33 and 64 grams for the two lower doses and a median of 52 grams for the higher doses of lead as compared with medians of 3 and 6 grams for the highest range of dosage of metallic lead in untreated pigeons, and a daily consumption of 23 grams in normal pigeons.

Magnesium sulphate. This was administered to 3 pigeons in a concentration of 0.66 per cent in food on the basis of a daily food consumption of 23 grams. This is the equivalent of about 0.433 gram per kilo per diem or a daily dose of about 26 grams for an adult of 60 kilos. Actually, however, the dosage received by the pigeons was somewhat less, i.e., a median of 0.304 gram per kilo (range 0.304 to 0.39 gram). This is due to the somewhat lower daily food consumption (20 grams). The

dosage of lead ranged from 0.74 to 1.3 gram per kilo, which corresponded to ranges of from 0.6 to 1.28 gram per kilo of lead in untreated pigeons the majority (83 per cent) of whom died. None of the pigeons receiving magnesium sulphate died. All had diarrhea, as indicated by the liquid nature of the stools. Presumably, absorption of the lead was diminished.

The values for the remaining criteria also showed favorable tendencies. That is, the maximal loss of body weight was 23 per cent (median), while in leaded pigeons without treatment this was 30 to 40 per cent. The maximal loss of weight appeared at the end of 23 days, which was practically the same (15 to 26 days) for untreated leaded pigeons. The median daily food consumption was 20 grams, which was much greater than the food consumption (3.8 to 6 grams) in leaded pigeons and compared very favorably with 23 grams in normal pigeons. The time of recovery was somewhat more prolonged (23 to 70 days; median 57 days) as compared with 26 and 45 days in the 20 per cent of untreated leaded pigeons (receiving similar dosage of lead) that recovered. On the whole, magnesium sulphate appeared to be beneficial in the treatment of chronic lead poisoning in pigeons.

Calcium sulphide. This was also beneficial as indicated by the absence of mortality with a dosage of lead (0.8 to 1.13 grams per kilo) which caused death in the majority of untreated pigeons previously reported (1). The daily dosage of calcium sulphide ranged from 0.1 to 0.19 gram per kilo equivalent to a daily dosage of about 6.0 to 11.4 grams for an adult of 60 kilos. Recovery from the lead effects occurred in from 22 to 47 days; the maximal loss of body weight was very small, ranging from 1.5 to 3.7 per cent and this appeared at the end of 9 to 25 days, the first appearance of loss of body weight being demonstrable at the end of about 4 days after administration of the lead. The median daily food consumption was 21 grams (range 15 to 27 grams) which compares favorably with the normal food consumption of 23 grams and was much greater than that (median of 3 to 6 grams) of untreated pigeons receiving a corresponding dosage of lead alone (0.6 to 1.83 grams). In fact, all of the factors by

which the therapeutic effects were judged were beneficially influenced. That is, loss of body weight was much less, and the progress of poisoning as indicated by the time of appearance of loss of body weight was slower, and the appetite of the pigeons was not markedly impaired as compared with untreated pigeons previously reported. The values for the different criteria have been sufficiently indicated in the sections on iodide and magnesium sulphate and need not be repeated here.

The results obtained with the therapeutic agents that were tried, namely, sodium iodide in food and water, and magnesium sulphate and calcium sulphide in food, justify the conclusion that they are beneficial in the treatment of chronic lead poisoning of pigeons receiving fatal doses of metallic lead. This agrees, in general with clinical results from these agents in the treatment of chronic lead poisoning in man. Iodide in water was somewhat more efficient than iodide in food, since the pigeons treated with the medicated drinking water survived larger doses of metallic lead than those receiving the iodide in food. Magnesium sulphate and calcium sulphide were about as efficient as iodide in water as judged by the complete recovery from corresponding doses of metallic lead. As for the remaining criteria, which were used in judging the therapeutic effects of the agents used, the discussions of the individual agents indicate that there were beneficial influences in several directions.

The experiments did not attempt to solve the mechanism of action of the different therapeutic agents although certain results that were obtained are suggestive. Magnesium sulphate acted as a cathartic and presumably prevented absorption of the lead. It is probable that the iodide and sulphide acted in part, at least, by rendering the liberated lead insoluble, and, therefore, unabsorbable. Evidences for this were obtained from experiments *in vitro* which indicate an inhibitory influence of the therapeutic agents that were used on the solubility of metallic lead (shot).

Inhibitory influence of iodide, magnesium sulphate and calcium sulphide on solubility of metallic lead

The experiments were carried out as follows: Definite quantities (about 0.13 gram) of metallic lead (weighed shot) were placed into vials containing 10 cc. of (a) 0.2 per cent hydrochloric acid containing sodium iodide 0.11 to 0.44 per cent, magnesium sulphate 0.66 per cent, calcium sulphide 0.11 to 0.44 per cent, and sodium chloride 0.11 to 0.44 per cent, and (b) into mixtures of pigeon food containing the same concentration of hydrochloric acid and the different salts as in (a), and (c) in aqueous solutions of the same concentrations of the salts without hydrochloric acid. The vials were stoppered; placed into an incubator at 38°C., and the shot were weighed once each week for 5 weeks in the majority of, and for 6 weeks in the remaining, experiments. The loss of lead was expressed as percentage dissolved at the end of each week. All told 6 experiments were made. The results that were obtained are illustrated by those of two experiments with the highest concentrations of the different salts in 0.2 per cent hydrochloric acid and 0.2 per cent hydrochloric acid and food, and are presented in the form of curves in the accompanying figure. The results of the remaining experiments with lower concentrations of the salts, and in water without hydrochloric acid, showed essentially the same tendency, differing only in some cases as to the percentage of lead dissolved. They will be omitted to conserve space. The discussion of results in the accompanying figure will suffice for all the results.

It is seen (part A of the accompanying figure) that sodium iodide, magnesium sulphate and calcium sulphide considerably lowered the solubility of metallic lead in 0.2 per cent hydrochloric acid alone, while sodium chloride increased the solubility during the entire period of observation, though much less at the end of the fifth week. Presumably compounds of lead corresponding to the anions of the therapeutic agents (salts) used were formed, namely, the chloride, sulphide, sulphate and iodide of lead. Of these the lead chloride is most soluble. The influence of sodium

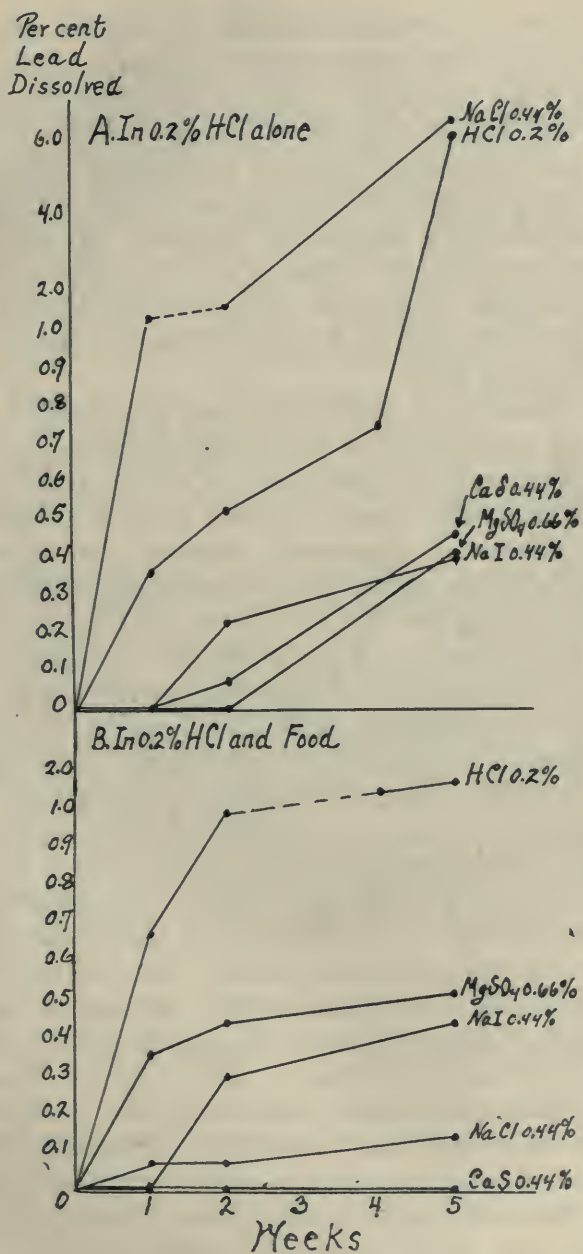


FIG. 1. THE COMPARATIVE SOLUBILITY OF LEAD IN LOW CONCENTRATIONS OF VARIOUS SALTS IN 0.2 PER CENT HYDROCHLORIC ACID (A), AND IN 0.2 PER CENT HYDROCHLORIC ACID AND FOOD (B), IN VITRO AT 38°C.

chloride, that is, increased formation of soluble lead chloride, correlates well with the greater toxicity of lead chloride than other lead compounds previously reported (2); with the therapeutic inefficiency of sodium chloride as compared with sodium iodide, sulphide and magnesium sulphate reported in this paper. Or, in other words, the beneficial therapeutic effects of sodium iodide, calcium sulphide and magnesium sulphate may be referred to their inhibitory solvent influence on the metallic lead, and, therefore, lessened absorption, while sodium chloride facilitates solution and, therefore, facilitates, or does not retard, at least, the absorption of lead.

In the presence of food containing hydrochloric acid and the different salts (part B of accompanying figure) the inhibitory influence of calcium sulphide was even more marked than in the same mixture in the absence of food (part A of same figure), while the effects of magnesium sulphate and sodium iodide remained practically unchanged; the inhibition of solubility being less marked during the first two weeks, and about the same at the end of five weeks. Sodium chloride produced a different effect in the presence of food than without it, namely, a marked inhibition in hydrochloric acid and food as compared with augmentation of solubility in hydrochloric acid alone. The solubility of lead in hydrochloric acid and food containing iodide was considerably retarded at the end of five weeks, amounting to 1.8 per cent as compared with 6 per cent in hydrochloric acid alone at the end of the same period. The tendency of these results with food is in line with those of Carlson and Woelfel (3) who found that the presence of protein (milk) retards the solution of lead carbonate in hydrochloric acid and gastric juice.

The results on solubility of metallic lead in vitro under different conditions and body temperature (38°C.) justify the conclusion that the therapeutic agents which were used in the treatment of chronic lead poisoning of pigeons receiving metallic lead, namely, sodium iodide, magnesium sulphate and calcium sulphide, markedly lessen the solubility of lead in 0.2 per cent hydrochloric acid alone and when it contains food.

CONCLUSIONS

1. Using the following criteria; mortality, maximal loss of body weight and its time of appearance, the first appearance of loss of body weight, time of recovery, and daily food consumption, the therapeutic efficiency of some agents in current use for the treatment of chronic lead poisoning was tested in experimental chronic poisoning of pigeons by metallic lead.

2. By this method of study, and using sodium chloride as control, the daily administration of sodium iodide in food and drinking water, and of magnesium sulphate and calcium sulphide in food, in doses corresponding to medium and large therapeutic doses, was found to be beneficial.

3. This agrees generally with the clinical results obtained with these agents in the treatment of chronic lead poisoning in man.

4. The mechanism of action of sodium iodide and calcium sulphide is not settled. The beneficial effects of magnesium sulphate are due to its cathartic action, in part, at least.

5. The solubility of metallic lead in 0.2 per cent hydrochloric acid and in 0.2 per cent hydrochloric acid and pigeon food in vitro at body temperature (conditions analogous to those in the gizzard) is markedly reduced by sodium iodide, magnesium sulphate and calcium sulphide.

6. Therefore, the beneficial therapeutic effects of these agents in the lead poisoning of pigeons are attributed partly to the diminished solubility of the lead and, therefore, diminished absorption.

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COMPARATIVE TOXICITY OF METALLIC LEAD AND OTHER HEAVY METALS FOR PIGEONS

P. J. HANZLIK AND ELIZABETH PRESNO

From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco

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In connection with previous studies (1, 2) of poisoning from metallic lead in pigeons there was an opportunity to test the toxicity of a number of the more important heavy metals. As far as we know, this has not been done previously on pigeons and to no extent on other species. Brouardel (3) states that Solles in 1881 found pigeons very sensitive to inhalations of metallic mercury, the symptoms being of a nervous nature. He denies poisoning in man by metallic copper and reports that feeding experiments on dogs by Drouard and Burcq were negative. Metallic lead is readily liberated in the pigeon's gizzard, causing prompt toxicity, and death with higher doses. The liberation in the gizzard depends mainly on the solubility of the metal in the digestive juices, and grinding capacity of the gizzard. A priori, therefore, the toxicity of various metals would be more or less proportional to their solubility. However, after absorption the toxicity would depend on the specific toxicity of the metallic ion and the concentration passing through the tissues.

The present paper presents briefly the results obtained with 12 different heavy metals. As compared with those on lead previously reported, they show that none of these metals is as toxic as lead even in doses up to about four times the fatal dose of metallic lead for the majority of pigeons.

METHOD

The method of study was the same as that of the first paper (1) of this series. Briefly, adult pigeons of about 350 grams body

weight were used. The experiments were conducted quantitatively. The pigeons were weighed regularly; their daily food consumption was determined and all changes were carefully noted until the body weight recovered to the previous level when the experiments were stopped. At least 3 pigeons were used with each metal. The following metals were studied; aluminium, antimony, bismuth, cadmium, copper, iron, manganese, mercury, nickel, silver, tin and zinc. The purest obtainable metals were used in granular form or pellets. They were introduced directly into the gizzard. The results obtained are presented in the accompanying table.

RESULTS

It is seen that out of the 42 pigeons that were used and treated with 12 different metals only 1 died, namely, 1 pigeon out of the 3 that received tin. The death in this particular pigeon may have been accidental, since another pigeon which received a still higher dose of tin recovered promptly. The dosage of the different metals used ranged higher than that used with metallic lead, that is, up to about four times the fatal dose of metallic lead for the majority of pigeons. In no case did death occur from a dosage equivalent to the highest fatal dosage (2.28 grams per kilo) of metallic lead that was used in a previous study. In fact, this maximal dosage was exceeded without mortality in 4 pigeons, receiving 4 different metals, namely, iron (3.32 grams per kilo), manganese (2.7 grams per kilo), nickel (2.69 grams per kilo) and tin (3.0 grams per kilo). This indicates the relatively high toxicity of lead as compared with the various heavy metals that were studied.

As far as the remaining criteria of poisoning are concerned, e.g., maximal loss of body weight, and its time of appearance, the first appearance of loss of body weight, daily food consumption and time of recovery, these were all rather variable, but in no case were the changes marked enough to indicate a greater toxicity than that of metallic lead. This is not due to incomplete action, since the pigeons were observed until recovery to the original body weight (before administration of the metal) occurred as in all the studies of poisoning by lead previously reported.

The majority (2) of the pigeons receiving aluminium and half (2) of those receiving cadmium showed no loss of body weight at any time. The remaining pigeons receiving different metals showed variable though relatively small losses in body weight, ranging from no loss to 21 per cent (maximal loss), except the single pigeon that died and received tin (32 per cent). The median percentage loss (maximal) of body weight in all pigeons was about 8 per cent. In other words, the degree of poisoning by the metals that were studied and as indicated by the maximal loss of body weight was much less than that by metallic lead, which produced a maximal loss of 30 per cent (median) with a range of dosage (0.16 gram to 0.5 gram per kilo) that was survived by the majority of pigeons and 15 per cent to 24 per cent (medians) with doses (ranges of from 0.6 gram to 2.28 grams per kilo) which were fatal to the majority.

The time of appearance of the maximal loss of body weight varied greatly, i.e., a range of from none to 36 days, for all pigeons receiving the twelve different metals. There was also marked variability in groups of pigeons receiving individual metals. For instance, in the case of nickel, the maximal loss of body weight in the 3 pigeons that were used appeared in from 6 days to 136 days. Therefore, it is impossible to correlate this factor with that of lead poisoning in which the time of appearance of maximal loss of body weight was more uniform, i.e., a range of 15 to 24 days (median) in all pigeons receiving fatal and non-fatal doses. The first appearance of demonstrable loss of body weight was from the end of the second to ninth day after administration of the metals, that is, somewhat later than the first appearance in leaded pigeons (following to fourth day).

The daily food consumption was not markedly impaired, ranging for all pigeons from 13 grams to 31 grams (medians) which compares favorably with the median daily consumption of 23 grams for normal pigeons. On the other hand, the daily food consumption of pigeons receiving non-fatal and fatal doses of metallic lead was more markedly reduced, amounting to a median of 16 grams for the non-fatal and medians of 3 to 6 grams for the fatal doses. This is further evidence that metallic lead

TABLE
Effects of poisoning by various heavy metals administered to pigeons

METAL	NUMBER OF PIGEONS USED	BODY WEIGHT grams	DOSAGE PER KILO ADMIN- ISTERED	MORTALITY	TIME OF RECOVERY**	MAXI- MAL LOSS OF BODY WEIGHT		TIME OF AP- PEAR- ANCE OF MAXI- MAL LOSS OF BODY WEIGHT		FIRST APPEAR- ANCE OF LOSS OF BODY WEIGHT	MEDIAN DAILY FOOD CONSUMPTION
						percent	days	days	days		
Aluminium.....	3	463	0.864	None	13	21	2	2	2	29	
		291	2.75			None	None	None	None	27	
		292	2.77			None	None	None	None	27	
Antimony.....	4	303	0.995	None	64	8.2	19	5	5	21	
		346	0.87			4.6	3	3	3	20	
		381	1.57			4.4	14	3	3	20	
		415	1.20			5.3	2	2	2	19	
Bismuth.....	4	356	1.12	None	Observed 12 days	None	None	None	None	19	
		357	1.12			2.0	8	8	8	17	
		364	2.23			1.4	4	4	4	19	
		305	1.10			11.8	27	15	15	21	
Cadmium.....	4	327	1.22	None	Observed 8 days	None	None	None	None	22	
		353	2.23			None	None	None	None	22	
		323	2.53			10.8	117	3	3	18	
		280	1.13			18.6	11	6	6	16	
Copper.....	3	285	1.2	None	15	5.2	11	6	6	15	
		313	1.3			11.2	12	9	9	14	
		314	2.56			None	None	None	None	Undetermined	

Iron.....	4	344	1.01	None	Incomplete end of 27 days 16	5.1	27	6	17
		411	0.973	None		1.7	9	2	24
		322	2.48	None		3.0	8	3	31
		241	3.32	None		None	None	None	20
Manganese . . .	3	411	0.973	None	Incomplete end of 130 days Observed 10 days 10	12.4	7	3	24
		394	1.01	None		None	None	None	15
		298	2.70	None		3.1	6	3	31
Mercury.....	3	347	3.0	None	19 11 8	4.9	15	5	32
		364	1.4	None		3.8	8	5	28
		300	0.68	None		2.3	5	5	24
Nickel.....	3	472	0.947	None	Incomplete end of 182 days 47 51	8.0	136	5	24
		297	2.693	None		16.5	6	3	13
		339	2.36	None		11.6	10	3	24
Silver.....	3	652	0.621	None	48 18 None (observed 88 days)	10.6	27	2	28
		379	0.53	None		5.6	10	4	21
		383	1.56	None		6.8	88	2	22
Tin.....	3	292	1.20	None	27 None 7	8.2	22	6	18
		300	2.66	Died*		32.0	17	3	13
		266	3.00	None		4.0	3	3	21
Zinc.....	5	298	1.20	None	18 21 12 17 11	15.1	6	6	17
		450	0.91	None		3.3	19	3	23
		344	1.17	None		0.6	7	7	23
		357	2.24	None		3.0	5	5	19
		408	1.74	None		2.8	7	4	23

* End of 17 days.

** Judged by recovery of body weight to previous level.

causes greater toxicity and more sickness in pigeons than aluminium, antimony, bismuth, cadmium, copper, iron, manganese, mercury, nickel, silver, tin and zinc.

The time of recovery also varied greatly. It was incomplete in 4 pigeons receiving different metals, namely, at end of 27 days after a dosage of 1.01 grams per kilo of iron; at end of 130 days from a dose of 0.973 gram per kilo of manganese; at end of 182 days from a dose of 0.947 grams of nickel and at end of 88 days from a dose of 1.56 grams per kilo of silver. On the other hand, the remaining pigeons (usually 2) which received higher doses of each of these same metals recovered completely in shorter periods of time. This leaves 28 pigeons which recovered anywhere from 7 to 156 days after administration of the different metals and only 1 pigeon that did not recover out of the entire lot of 42 pigeons used in the study. Pigeons in a previous study which received doses of metallic lead corresponding to the doses to the different metals used in this study did not recover at all. Therefore, there is no good basis for comparison of the time of recovery in these with the leaded pigeons. With much smaller doses of lead the time of recovery among the majority of pigeons which received non-fatal doses was a median of 56 days (range 41 to 150 days).

CONCLUSIONS

1. The following heavy metals administered to 42 pigeons up to four times the fatal dose of metallic lead for the majority of pigeons previously reported were variably and moderately toxic, namely, aluminium, antimony, bismuth, cadmium, copper, iron, manganese, mercury, nickel, silver, tin and zinc. Out of the entire lot of 42 pigeons used only 1 pigeon died, namely, 1 that received a medium dose of metallic tin.

2. Therefore, metallic lead is more toxic and plumbism is more or less a specific toxicity in the sense that characteristic symptoms occur rather promptly, and mortality is higher with smaller doses than with other heavy metals.

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THE BIOLOGICAL ACTION OF POTASSIUM AND ITS RADIO-ACTIVITY

H. ZWAARDEMAKER

Utrecht, Holland

Potassium exercises a general ionic influence and a specific atomic influence.

With regard to ionic action the potassium-ion is similar to the other univalent ions, which occur constantly in the tissues; by its specific atomic properties it possesses a more prominent significance. One of its specific properties is radio-activity. No other element of the organism possesses that property and therefore it should be considered first of all.

In doing so we were able to establish two laws in a considerable number of animal systems (1):

1. Potassium can be substituted by all the other radio-active atoms in radio-equivalent quantities.

2. An antagonism manifests itself between the elements emitting alpha-rays and those emitting beta-rays.

Either law is independent of the other (3). They have a broad field of applicability to many functions.

In a recent issue of the Journal A. J. Clark has criticized my investigations and he arrives at the conclusion (3).

Uranium and thorium can compensate to a certain extent for lack of potassium in the frog heart under a certain narrow range of conditions, but under all other conditions in the frog's heart, and under all conditions in other tissues there is no evidence at all that uranium and thorium act as substitutes for potassium.

In the present article I will endeavor to extend Clark's "narrow range of conditions" for the heart, and also describe more fully the conditions for other organs.

It may be useful to give beforehand some remarks on the equiradio-activity of the doses in which the radio-active elements must be applied to ensure the effect of the substitution.

For the organs of poikilotherms the empirically established summer-doses in milligrams per litre of circulating fluid are:

K.....	5
Rb.....	10
U.....	0.5
Th.....	1
Ra.....	0.5×10^{-6}
Em.....	100 M.E.

The radio-activity of these amounts I take to be nearly equivalent, anyhow of the same order. That some of the elements are beta-rays, others alpha-rays, may, indeed, complicate the comparison, but it does not render it "hopeless" as Clark asserts.

According to Lazarus (4) is the ionizing power of potassium stands to that of the beta-rays of radium in the ratio of about $4:10^9$. The ratio of the ionizing power of potassium and that of the alpha-rays of radium is $4:10^{11}$, for it is said that the ionizing power of radium for air depends for only 1 per cent upon its beta-rays. The radium used in these experiments was in equilibrium with its transition products. But the action of pure radium with which we have to do when we distribute atoms of radium over a large extent of tissue, is weaker than radium that is in equilibrium with its products. This renders the ratio for potassium more favorable.

Besides the point in question it is not the ionization of air, but an action upon the cells, which for the present we will suppose to depend upon the kinetic energy of the particles shot out. In doing so it should be remembered that the potassium-rays have a power of penetration 8 times stronger than the beta-rays of radium, so that their kinetic energy is 4 times greater.

Again, radium-atoms are about 6 times heavier than potassium-atoms. In a certain weight, say 1 mgm. they are 6 times smaller in number. The chances of colliding with a cell, therefore, are also 6 times smaller.

Now the alpha-radiation of pure uranium may readily be compared with the alpha-radiation of radium. Rutherford and Geiger (5) found that 1 gram of uranium emits $2.37 \cdot 10^4$ alpha-rays per second, pure radium, however, 3.410^{10} or 1.410^6 times more.

Considering the heat produced by uranium and by thorium the energy of the latter must be deemed 2.7 times weaker than that of uranium. The dose to be taken might, therefore, be supposed to be 2.7 times larger.

The result entitled us to speak in our experiments of radioequivalent doses. When afterwards the summer-doses became known, the agreement between the calculated and the experimental values was completely satisfactory. We maintain, therefore, that, when the total kinetic energy emanating from the radiation is taken as standard, the law of the radio-equivalent substitution must be considered to be properly established.

The question being one of peculiar interest I requested one of my co-workers to make a series of determinations in the field of our inquiry with another animal, viz., the *Petromyzon fluviatilis*, and that for the entire heart in situ. The inflow cannule is therefore slipped into the right vena cava, the outflow cannule into the a. branchialis. The average minimum dose per litre of perfusion fluid (which contains 240 mgm. CaCl_2 and of a pH 7.6) was 15 mgm. KCl or 5 mgm. uranyl nitrate or 10 mgm. thorium nitrate. The maximum dose which arrested the heart beat, was 1 gram KCl or ± 50 mgm. uranyl nitrate or ± 100 mgm. thorium nitrate. An average amount of, say, 100 mgm. KCl, 10 mgm. of uranyl nitrate, 20 mgm. of thorium nitrate, may be considered as the optimal dose. With this amount the heart beats usually continue for a hour or more, without any notable deviation from the normal pulsation. Also the electrocardiogram is unchanged at the beginning. Many details, which presented themselves will be published in the theses of Mr. Slooff and Mr. Zwaardemaker Jr. In the same way the heart of the eel, frog, turtle, etc., can be brought to a state in which when it is perfused in situ, it gives perfectly normal, complete pulsations for many hours. For that only the conditions and the optimal dose need to be found.

Clark draws the conclusion that the pulsation during the replacement of potassium by uranium or thorium is, properly speaking, not a normal automatic one but a pulsation brought about by the stimulating action of uranium and thorium. I quite agree with him on this point, if he will also consider the normal automatic beat as a pulsation brought about by the stimulating action of potassium.¹ In point of fact there are two kinds of automaticity:

1. The natural automaticity in the presence of potassium, which may be imitated by rubidium.

2. An artificial automaticity revealing itself when potassium is replaced by radio-active elements with alpha-radiation.

Broadly speaking the natural and the artificial automaticity run parallel as they resemble each other in many respects: in frequency, in conductivity, in mechanical excitability, in emission of energy, in electric phenomena.

Figure 1 illustrates perfusion alternately with 250 mgm. of potassium chloride per litre, and with 25 mgm. of uranyl nitrate per litre. In the morning the interchange took place without any disturbance; in the afternoon a transient standstill occurred at the moment of interchange. The two automaticities differ only in details. We have been trying to trace these differences. Fundamental differences are, e.g., varying behavior towards the electric current, diathermy etc. Whereas the normal automaticity with the same frequency in its optimum point by application of electric stimulation, only shows a slightly increased frequency, the alpha-automaticity will be reduced to a standstill by precisely the same intensities. An additional striking difference was constituted by the simultaneous tonus of the heart muscle. In other respects however there are only quantitative, negligibly small differences.

It is no wonder, in fact, that the alpha-rays and the beta-automaticities are not completely alike. The radiation of

¹ Only one should not be led to suppose that every systole is produced by such a stimulus. It is the automaticity as a whole, the spontaneous rhythmic succession of alternate katabolism and anabolism in a metastable system, which is kept up by the occasional shocks of corpuscular radiation.

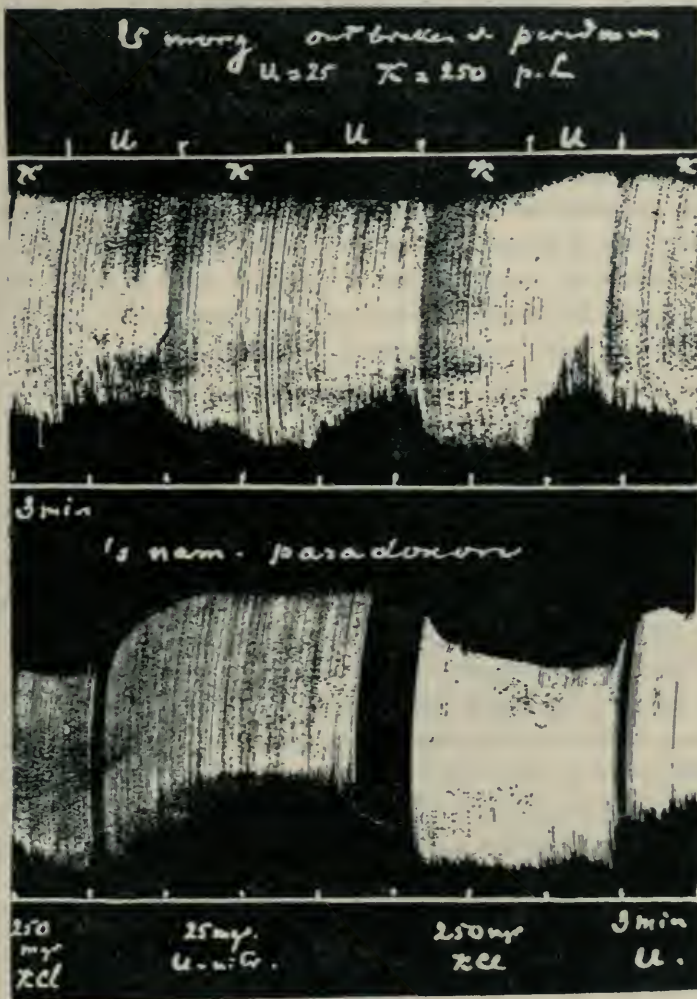


FIG. 1. FAILURE OF PARADOXON IN THE MORNING, APPEARANCE IN THE AFTERNOON, IN THE SAME FROG HEART

$K = 0.250$ gram potassium chloride per liter Ringer's fluid.

$U = 0.025$ gram uranyl nitrate per liter Ringer's fluid, when deprived of potassium.

Time—intervals, three minutes.

The presence of uranium in the perfusion causes a slight tonicity in the muscle. When potassium appears in the fluid this tonicity is lost immediately.

potassium or rubidium continually imparts negative charges to the system, the charges transmitted by the radiation of uranium, thorium, radium, ionium, emanation are always positive. Moreover the rate at which the charged ions fly through the excitable systems, is different. How could the result be reasonably expected to be the same?

Clark gives in his article a series of determinations of the considerable loss of potassium by the depot in the heart-cells during the substitution experiments. Jannink had already demonstrated in my laboratory a deprival of potassium from the frog's heart which had been perfused with potassium-free Ringer's solution to a quantity of 1 mgm. KCl per litre of circulating fluid, but such a great liberation of potassium as Clark found, could not be suspected. Neither could it be surmised from Mitchell and Wilson's tests on potassium.

One-third of the potassium present in the cells is cleared away in two hours and half of it in six hours. Clark's finding is of paramount importance for radio-physiology, since it makes superfluous our hypotheses tending to explain the inactivity of the permanent potassium-depot. Evidently it now comes to this: When feeding a frog's heart for half an hour with Ringer's solution without potassium, the potassium in the cells will dwindle down to five sixths of the normal amount. With this the threshold is reached of the potassium-activity required to keep the automaticity going, and the function of the heart is arrested. The heart will recover its beats only when a more intense beta-radiation than is procured by the depot residue, is acquired through a fresh supply of potassium from the outside. When replacing potassium from the radio-active element with alpha-radiation a small amount should be applied at first to destroy the depot-radiation that has been left, and then an excess of it to restore the pulsation, now under the influence of positively charged rays, which are emitted with much lower velocity, but are, on the whole, much larger. This helps to explain our experience of 1918, when we found doses that were somewhat larger than those found through calculation.

It seems to me that the most important result of Clark's inquiry is to have rendered this view possible. A number of phenomena have been clarified by it, e.g., the influence which radio-active radiations appear to exercise on the vagus-effect.

Clark himself thinks differently of his finding. He remarks that the radiation of potassium has a high penetrating power, and he asserts that it reaches its half-value at the distance of 2 mm.

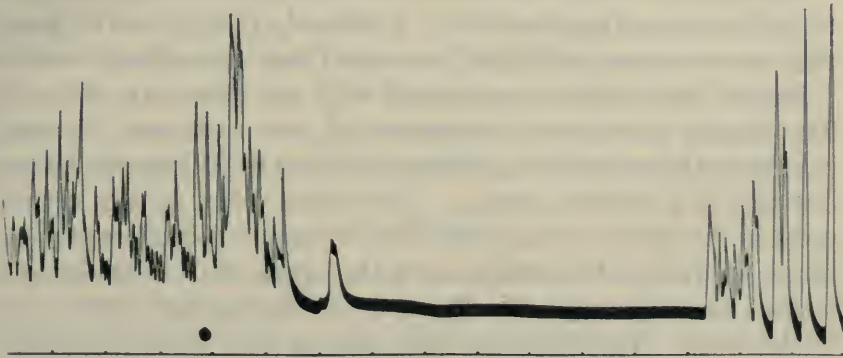


FIG. 2. CONTRACTIONS OF THE UTERUS OF A WHITE MOUSE

During half an hour the organ makes spontaneous pulsations with perfect regularity in a Ringer fluid, which is deprived of potassium and contains 0.03 gram thorium nitrate pro liter. The black spot signals the moment at which the thorium fluid is replaced by a normal Ringer fluid. My paradoxon is the consequence. Immediately the tonic of a thorium-state disappears. When the pulsations begin again, the relaxations are more complete, announcing the potassium-state of the organ. The frequency of the thorium pulsations was permanently greater than the frequency of the potassium-pulsations.

Time—intervals in minutes.

This is in reality only 1 mm.; according to the measurements made by W. E. Ringer and myself with the filtering aluminium film (half-value with 0.25 mm. thickness). However leaving this aside the question remains where the threshold of automaticity is still latent. When the perfusion is continued for longer than an hour, this latent automaticity will also have ceased. Probably we have then come entirely beneath the threshold of the potassium-rays. This way of looking upon the question is as justifiable as Clark's view. Why assume,

1. A specific stimulating chemical action of potassium
2. A corresponding chemical action of rubidium
3. A weaker chemical action of caesium
4. A specific stimulating chemical action of uranium
5. A specific stimulating chemical action of thorium
6. A specific stimulating chemical action of the constant current

all of which are conducive to the same effect, automaticity, and at the same time ignore the common possible cause?

Potassium and its substitutes have also proved to be of importance for some other functions. I will only refer to one of them, viz., the uterus-automaticity, because Clark also alludes to it.

Simple substitution-experiments with the uterus are difficult. The regular automatic contractions of the organ may, indeed, be arrested after perfusion, with a potassium-free Ringer's solution, but this is a tedious process. The fittest object for this experiment is the uterus-cornu of white mice, some weeks after parturition. In view of the feebleness of the organ the registration has to take place photographically, to avoid the friction of the point of the lever. The perfusion must also be very profuse and permanent. By simply refreshing the perfusing fluid a positive result cannot be obtained at all.

When there is sent through for four or five hours consecutively a potassium-free Ringer's solution at 38°C. containing a profuse amount of oxygen, the small organ has lost potassium enough to attain a condition of quiescence. Substitution of the removed potassium by uranium or thorium causes a recurrence of the weak regular contractions. An easier experiment is the following. The cornu of the uterus of a mouse is first brought into a Ringer's solution with 100 mgm. of KCl. After regular contractions have been going on for half an hour a Ringer's solution deprived of potassium but containing 30 mgm. thorium nitrate is admitted without interruption of the perfusion. Generally a change in the rhythm of the contractions soon reveals itself. Their frequency is slightly increased. Also a slight tonus is developed in the organ. After this process has continued for half an hour the Th-solution is again replaced in the same way by the K-solution. Then my paradoxon manifests itself, a momentary

standstill opening with a gradual lowering of the contractions and afterwards terminating in a similar gradual increase. The potassium-contractions having again proceeded for half an hour, the experiment may be repeated. Again the slight increase of frequency and the development of tonus can be observed on perfusion with the alpha-substances. Then the thorium-perfusion may be continued for an indefinite time and the automaticity by alpha-radiation may still be witnessed for an hour or longer. Owing to the tonus the excursion of the contractions of an alpha-automaticity are never as high as those of the natural automaticity. In most cases the experiment has to rest satisfied with somewhat smaller, but quite normal, uniform contractions, succeeding each other in a tenaciously preserved tempo.

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ON THE DETECTION OF BENZENE IN CADAVERS¹

ALEXANDER O. GETTLER

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The properties upon which the identification of benzene depends are mainly physical, such as the boiling point, refraction and specific gravity of the benzene itself or of some of its derivatives obtained by bromination, nitration and sulphonation. At least a few drops of the pure water-free benzene are deemed necessary for these tests. There are no color or precipitation reactions for dilute aqueous solutions of benzene.

In the analysis of cadavers only very small amounts of benzene (0.050 to 0.150 grams) may be recovered. This amount is soluble in the volume of water which comes over with the benzene during distillation and, therefore, none of the benzene settles out as a separate layer. Because of the difficulty in obtaining a requisite amount of water-free benzene and the lack of any test for benzene in dilute aqueous solution, very little work had been done on this subject until 1915, when G. Joachimoglu² demonstrated for the first time the presence of benzene in the organs of animals. Nothing of importance in this connection has been accomplished since then. For this reason the following case with laboratory findings being the first one on record should be both interesting and valuable for future medico-legal work.

R.F.M., a youth of sixteen years, was found lying face down on the floor of a small garage. He was discovered there at about 12:10 p.m. by the wife of the owner, who at once dragged him into the air. Upon questioning both the man and his wife, it was learned that the deceased

¹ From the Chemical Laboratories of the Department of Pathology, Bellevue Hospital, and of the Chief Medical Examiner's Office, City of New York.

² *Biochemische Zeitschrift*, 1915, lxx, 93-104.

had entered the garage at 9:30 a.m. and found the owner filling cans with a mixture containing benzene; some of the solution had been spilled on the floor. The boy remained in the garage only a short while and the owner left at about 9:45 a.m. No information could be obtained regarding the youth's whereabouts from 9:45 a.m. to 12:10 p.m., at which time he was found dead. It is believed that he returned shortly after the owner had left the place and that he started to fill some of the cans with the material. The doors of the garage being closed, he was overcome by the benzene fumes and fell to the floor. The boy was removed to the Kings County Morgue and an autopsy was performed the following morning by Dr. C. B. The autopsy findings were negative except that there was marked congestion of all the organs.

Method used in the chemical analysis of the organs: 300 grams of tissue were ground up (while in a frozen condition) and placed in a distilling flask. To this, 300 cc. of water were added, thoroughly mixed and acidified with a few drops of sulphuric acid. The flask was then connected with a long well cooled condenser, the distal end of which was immersed in about 25 cc. of C.P. CCl_4 in an Erlenmeyer flask which was packed in ice. The flask and contents were then heated on a boiling water bath. A current of steam was passed through the material to accelerate the distillation of the benzene. This distillation was continued for two hours, after which the distillate and CCl_4 layer were placed in a separatory funnel, well shaken for a few minutes, and allowed to stand. When the two layers had separated, the lower one (CCl_4), which contained most of the benzene, was placed in a clean, dry flask and stoppered. The aqueous layer was shaken out in a similar manner three or four times, using fresh CCl_4 each time. These several portions were added to the first CCl_4 portion in the flask, which then contained practically all of the benzene. To this tetrachloride solution of the benzene were added about 10 cc. of a 2:1 mixture of fuming nitric-sulphuric acid, and thoroughly shaken. This process converted the benzene to dinitro-benzenes. The entire mixture was then placed in an evaporating dish and the CCl_4 carefully evaporated on the water bath. The residue, consisting of dinitro-benzenes and nitrating acids, was allowed to cool and was then treated with about 50 cc. of water and neutralized with sodium hydroxide. This solution of the dinitro-benzenes was extracted in a separatory funnel several times with fresh portions of ether. (The ether takes up the dinitro-benzenes.) On evaporating the ether, the nitro-benzenes were left as a yellow residue with their characteristic odor. This nitro-benzene

residue was weighed and from the result (calculating as dinitro-benzene) the amount of benzene originally present in the organ was computed.

After determining its weight, the residue may be used for further confirmatory tests. A small portion of it (about $\frac{1}{3}$) is taken up in about 5 cc. of absolute alcohol and made alkaline with 3 drops of 30 per cent sodium hydroxide. To this is added twice the volume of 1 per cent fructose solution. A violet color results, then gradually fades. This test reacts positively only with ortho or meta dinitrobenzene, but these dinitro compounds are always formed in the nitration. No other nitro compounds give this reaction.

Amount of benzene recovered

AMOUNT USED	KIND OF TISSUE	WEIGHT OF DINITRO- BENZENE	BENZENE	BENZENE PER 100 GRAMS OF TISSUE
<i>grams</i>		<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
300	Liver	65	30.2	10.1
300	Brain	124	57.5	19.2
150	Blood	21	9.7	6.4
100	Fat	Trace*	Trace*	Trace*
500	Lung	97	45	9.0
80	Stomach contents	Absent	Absent	Absent
150	Intestinal contents	Absent	Absent	Absent
80	Spleen	Trace*	Trace*	Trace*
150	Kidney	Absent	Absent	Absent
150	Heart	8	3.7	2.4

* Weight of the dinitro-benzene was less than 2 mgm. It responded positively to both the alpha naphthol and the isonitrile reactions.

The remainder of the residue is taken up with about 10 cc. of water in a test tube, to which are added about 2 grams of zinc dust and concentrated hydrochloric acid, drop by drop, until a fair (not too violent) reaction ensues. If the mixture gets too hot, it is cooled by immersing the test tube in cold water. This reduction is continued for about ten minutes and results in the production of phenyl diamine. A portion of the reduced material is made alkaline with alcoholic sodium hydroxide, one drop of chloroform is added, and the mixture is heated. The typical disagreeable and irritating odor of phenyl isonitrile is easily perceptible. To another and major portion of the mixed amines are added a few drops of 10 per cent NaNO_2 (diazotization), then sodium carbonate solution (until a precipitate of zinc carbonate is formed), and then 3 cc. of 0.1 per cent alkaline solution of alpha naphthol. An

intense red-brown color (delicate to 1:100,000) indicates the presence of an aromatic amine. (The latter test is more delicate than the fructose test, but it is not specific.)

Cyclic compounds, such as nitro-benzene, phenol, picric acid, etc., will yield products under the above mentioned treatment that also give a positive reaction with the fructose, alpha naphthol or isonitrile reaction. It is, therefore, imperative to test for these substances in all examinations for benzene.

To serve as controls, 20 human livers, 18 brains, 10 lungs, 15 kidneys and 5 blood samples were analyzed in a similar manner. These tissues were obtained at autopsy. The cause of death was through disease or shooting. Poisoning of any kind was excluded by a complete chemical analysis. In all of these 68 tissues, the above described tests were negative. A small amount of residue (0.3 to 1.2 mgm.) was obtained in each case on evaporating the ether. This amount (average 0.8 mgm.) should be subtracted from the weight of the dinitrobenzene obtained in poisonings of this kind.

A STUDY OF THE RATE OF DEPOSITION AND PATHS OF ABSORPTION OF STRONTIUM IN THE RAT¹

ETHEL MAY KINNEY AND E. V. MCCOLLUM

*From the Department of Chemical Hygiene, School of Hygiene and Public Health,
The Johns Hopkins University, Baltimore*

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Strontium belongs to the group of alkaline earths, and therefore is closely related chemically to calcium and magnesium. It has been frequently assumed that within the body, particularly in the skeletal system, strontium may be substituted for the two related elements. In connection with a study of the histological changes which result in the bones as the result of feeding strontium, we had an opportunity to examine the validity of certain statements in the literature regarding the behavior of this element (1). We have here extended our study to include data of several kinds relating to the rate of deposition of strontium in animals of different ages, and the paths by which strontium may enter the body.

In 1870 Papillion (2) endeavored to determine if the calcium of the bones could be substituted by strontium or magnesium. He fed one pigeon and two rats rice and gluten and a salt mixture. Just which salts of strontium and magnesium and the amounts of each which he fed are not given. He obtained the following results, and his conclusions were that the calcium of bone could be substituted by strontium or magnesium or other of the alkaline earths or earth metals.

¹ The data for this paper are taken from a Dissertation submitted by Ethel May Kinney for the degree of Doctor of Science to the Faculty of the School of Hygiene and Public Health, The Johns Hopkins University, June, 1922.

	PIGEON	RAT I	RAT II
Addition to food	Strontium	Magnesium	Aluminum phosphate
Duration of feeding	7 months	70 days	74 days
In bone ash	CaO, 46.75% SrO, 8.45%	CaO, 46.15% MgO, 3.56%	CaO, 41.10% Al ₂ O ₃ , 6.95%

In 1872 Weiske (3) fed 8 groups of rabbits one hundred days with hay and turnips. One was a control group which received no added mineral salts. Two groups each, one of grown animals and one of young animals, received, respectively, calcium phosphate, magnesium phosphate, and strontium phosphate. Weiske was not able to find a trace of strontium in the ash of these bones. He could find no appreciable change in the ash content of his experimental animals over that of the control group.

In 1874 König (4) questioned this work. He thought that Weiske probably got no change in the bone ash when supplementary salts were added because the food contained a sufficient amount of normal bone-building elements, and any excess of these normal elements or any additional and unnecessary ones were simply discarded by the animal body. He repeated Weiske's work, giving each of his animals 100 grams per day of wheat gluten, starch, saw-dust, and carrots. This diet is very low in its mineral content. He isolated very appreciable quantities of strontium from the bones of the strontium-fed animals. His chemical analyses are as follows:

DIET	DURATION	PERCENTAGE CONTENT IN BONE ASH			
		CaO	SrO	MgO	H ₃ PO ₄
	days				
Young rabbits (5 weeks old) normal diet . .		52.73		1.62	40.92
No. 1. Calcium phosphate	70	51.36		0.70	42.54
No. 2. Calcium phosphate	73	51.92		0.82	42.50
No. 3. Strontium phosphate	23	44.77	5.21	0.64	39.64
No. 4. Strontium phosphate	27	49.27	4.71		40.68
No. 5. Strontium phosphate	20	46.78	5.37	1.09	39.47
No. 6. Magnesium phosphate	31	51.60		1.48	39.51
No. 7. Magnesium phosphate	82	51.92		1.68	42.27

An interesting point to be observed in the above table is the smallness of the calcium content of the strontium-containing bones. The total percentage of the calcium oxide and a strontium oxide just about equals the percentage of calcium oxide in the normal bones. The data which we have obtained confirm König's results.

This criticism of König's led Weiske to repeat and extend his work on strontium but again he was unable to demonstrate any strontium in the bone-ash (5).

In his discussion of the literature pertaining to strontium-containing diets, Lehnerdt cites an experiment by Korsakov (6) in which Korsakov fed strontium to 2 pregnant animals, 1 for twenty-eight and the other for fifty days prior to delivery. The mother remained in good condition, but the young showed symptoms of congenital rickets. Korsakov was unable to find any strontium in the bone ash on chemical analysis.

Stoeltzner (7) examined the bones of a number of animals receiving a diet of horse flesh and fat with additions of strontium phosphate. Her results are summarized in the following table.

CALCIUM-POOR DIET CONTAINING $\text{Sr}_2(\text{PO}_4)_2$	WHOLE BONES		
	$\text{CaCO}_3 + \text{SrCO}_3$	CaCO_3	SrCO_3
Pregnant dogs	55.58	50.74	2.17
Young animals A and B	44.51	37.10	6.41
Young animal C	47.34	36.29	6.08
Normal bones		57.29	

With these diversified and conflicting data before us, and in the light of improved feeding and chemical technique, it seemed quite worth while to endeavor to answer the following questions concerning the deposition of strontium in the skeletal system of the rat.

1. Is strontium deposited in the bones of the body?
2. How rapid is this deposition?
3. Does the age of the animal affect this deposition?
4. Is strontium deposited when an animal is receiving an optimal amount of bone-forming elements?
5. Through what paths does strontium enter the skeletal system?

CHEMICAL PROCEDURE

McCrudden's method for the determination of calcium and magnesium in the presence of phosphates was, with some modifications, followed. Strontium was separated from the calcium and magnesium according to Treadwell and Hall (8). It is very necessary in this method of analysis that absolute alcohol and anhydrous ether be used for the separation of the calcium and strontium nitrates. Very small amounts of water in either of these reagents will dissolve the strontium nitrate.

It seems very likely that this may have been the cause for Weiske's not obtaining any strontium in his analyses. In his discussion of his methods he omits any statement to the effect that he used absolute alcohol and ether.

The possible errors in the separation of the calcium from the strontium in plant ash are discussed by Robinson, Steinkoenig, and Miller (9) of the United States Department of Agriculture. They say:

The determination of strontium in plant ash is not satisfactory. A large amount of calcium is always present, a fact which necessitates a large volume in which the precipitation is to be made. It seems probable that a considerable portion of the small amount of strontium present escapes precipitation in both the sulfate and oxalate precipitations. Further, some strontium nitrate is probably dissolved by the comparatively large amount of absolute ether-alcohol which must be used to dissolve the calcium nitrate and completely wash it from the strontium nitrate. An error in the other direction arises through the difficulty of washing the strontium nitrate free from the calcium salts.

Thus it seems that the possibilities for error in the determination of strontium are much greater for too low rather than for too high results.

DEMONSTRATION OF THE DEPOSITION OF STRONTIUM IN THE BONES

In order to determine if strontium is deposited in the bones, three rats were fed the following ration until they died. This ration shall be referred to as ration I.

Ration I

	<i>per cent</i>
Liver.....	20.0
Casein.....	10.0
NaCl.....	1.0
KCl.....	1.0
SrCO ₃	2.2
Butter fat.....	3.0
Dextrin.....	62.8

When the strontium of the above diet is replaced by an equimolecular amount of CaCO₃ (1.5 per cent) it is satisfactory in all respects for the nutrition of the rat. It contains the optimum amounts of the mineral elements, proteins of good quality, and the essential vitamin factors. Such a ration was made up and fed to control animals. This ration shall be referred to as ration II.

Ration II

	<i>per cent</i>
Liver.....	20.0
Casein.....	10.0
NaCl.....	1.0
KCl.....	1.0
CaCO ₃	1.5
Butter fat.....	3.0
Dextrin.....	63.5

On this ration rats grow and produce and rear young in a most satisfactory manner, and live to old age. Therefore, it is obvious that the only faults to be found with ration I are its low calcium content and the presence of strontium.

In all the following analyses of the bones, the bones were freed from adherent flesh by boiling the bodies of the animals for several hours. For "percentage of ash" determinations the bones were weighed, after being dried in an oven heated to 100°C. for six hours. The bones were then ashed in an electric muffle for one hour, and the residue weighed. The chemical analyses were made on the ash and percentages calculated in terms of this constituent. The results of this work are tabulated in table 1.

These results indicate not only that strontium is deposited in the bones, but that it is deposited in considerable quantity.

TABLE 1

Percentage of calcium and strontium obtained from the bones of rats on a diet containing 2.2 per cent of strontium carbonate (ration I)

DAYS ON DIET BEFORE DEATH	CALCIUM IN ASH	STRONTIUM IN ASH
53	31.68	7.25
65	31.33	7.48
62	31.61	7.46

RATE OF DEPOSITION OF STRONTIUM

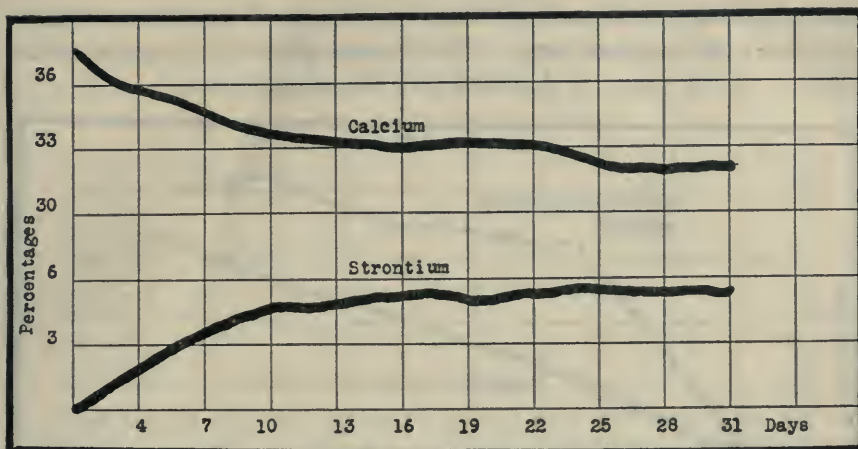
In order to determine the rate at which strontium is deposited, young animals were fed the above ration, and killed at three-day intervals. These results are tabulated in table 2.

TABLE 2

Effect on the composition of the bones of young rats receiving a diet containing 2.2 per cent strontium carbonate (ration I)

DAYS ON DIET	WEIGHT WHEN PUT ON DIET	WEIGHT WHEN KILLED	PERCENTAGE CALCIUM IN ASH	PERCENTAGE STRONTIUM IN ASH
0		80	36.63	0.00
4	89	85		2.61
7	63	59		4.04
10	92	79	33.84	4.41
13	102	115	33.49	4.50
16	101	110		5.25
19	100	126	33.28	5.10
22	96	124	32.34	5.35
25	93	116		lost
28	96	102		5.67
31	81	92	32.86	5.97

These data show that strontium is deposited very rapidly and regularly, and that as the strontium increases the calcium decreases. The total amount of ash remains essentially unchanged. This is graphically shown in the following curves.



THE EFFECT OF THE AGE OF THE ANIMAL ON STRONTIUM DEPOSITION.

In order to determine if the bones of mature animals were affected by strontium to the same degree as young ones, rats weighing between 150 to 180 grams were fed the strontium-containing diet. They were killed at four-day intervals. The results are tabulated below.

TABLE 3

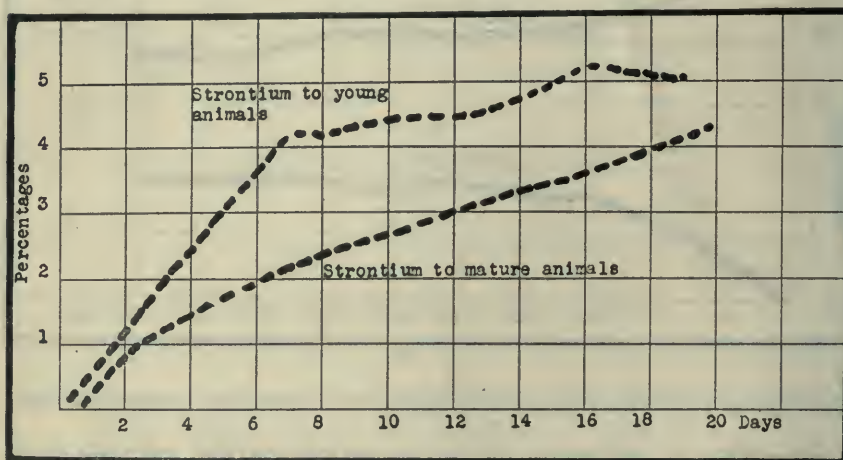
Effect on composition of bones of grown rats by feeding a diet low in calcium, high in phosphorus, and containing 2.2 per cent of strontium carbonate (ration I)

TIME ON DIET	PERCENTAGE OF ASH	PERCENTAGE OF CAL- CIUM IN ASH	PERCENTAGE OF STRONTIUM IN ASH
4 days	65.2		1.33
8 days	65.7		2.28
11 days	62.1	34.09	2.76
15 days	63.5	33.52	3.40
19 days	62.8	34.35	4.07
10 months*	66.1	37.7	0.0

* Normal bone (stock animals).

Again the strontium of the ash was increased and the calcium decreased as the feeding continued, but these changes did not take place so rapidly as in the younger animals. This might be expected, since, in general, the young and rapidly metab-

olising tissue is more susceptible to a change of condition of any sort than the mature one. The curves afford a graphic comparison of the rates of deposition of the strontium.



Since such a high percentage of strontium was found in the bones of rats which had received strontium for only four days, it was thought that it would be of interest to determine the amounts in one, two, three, and four days, respectively. Rats weighing between 100 to 130 grams were used. The following results were obtained.

TABLE 4

Percentages of calcium and strontium found in the bones of rats fed the strontium containing diet for short periods of time (ration I)

DAYS ON DIET	PERCENTAGE OF ASH	PERCENTAGE OF CALCIUM	PERCENTAGE OF STRONTIUM
1	63.96	36.76	0.67
2	64.06	36.68	0.78
3	60.22	36.31	0.98
4	61.79	36.38	1.21

EFFECT OF STRONTIUM FEEDING WHEN THE ANIMAL IS RECEIVING AN OPTIMAL AMOUNT OF BONE-FORMING MINERALS

In order to determine if König's suggestion was true that strontium might not be deposited in the bone if a sufficient amount of calcium was supplied in the diet, 1.5 per cent of the

dextrin of ration I was replaced by CaCO_3 . Or, in other words, 2.2 per cent of SrCO_3 was added to the optimal diet, ration II. The ration was made up as follows:

Ration III

	<i>per cent</i>
Liver.....	20.0
Casein.....	10.0
NaCl.....	1.0
CaCO_3	1.5
KCl.....	1.0
SrCO_3	2.2
Dextrin.....	61.3
Butter fat.....	3.0

This ration was fed to mature rats and the rats and the ash analyzed exactly as in the preceding experiment. The data in table 5 were obtained.

TABLE 5

Effect on the composition of the bones of mature rats receiving a diet containing 1.5 per cent of calcium carbonate and 2.2 per cent of strontium carbonate (ration III)

DAYS ON DIET	WEIGHT WHEN PLACED ON DIET	WEIGHT WHEN KILLED	PERCENTAGE CALCIUM IN ASH	PERCENTAGE STRONTIUM IN ASH
0		150	36.32	0.0
5	148	141	34.13	1.76
9	162	155	34.60	2.19
13	167	186	36.79	2.17
17	183	216	34.57	4.04
21	178	218	34.98	4.90
25	170	194	35.76	3.25

Several interesting facts are evident from these data. First, König's premise concerning the effect of sufficient bone-building elements was wrong. The presence of a sufficient amount of calcium in the diet does not inhibit the deposition of strontium. The rate of deposition does not seem to be uniform in all animals, but, in general, it appears to be about the same as in the mature animals receiving the low calcium diet (ration I). On the other hand, the calcium makes a small drop in the first five days, but thereafter tends to increase slowly toward the normal. The data would seem strongly to indicate that strontium behaves independently of calcium as a constituent of the

bone, and does not replace it physiologically as a bone-building element.

THE PATHS THROUGH WHICH STRONTIUM ENTERS THE BODY

In order to determine if strontium can enter the body through the placenta and through the milk of the mother, as well as by way of the alimentary tract, two series of experiments were carried out. In the first nursing rats of mothers receiving the strontium carbonate diet (ration I) were killed at varying periods and the entire bodies ashed. The amounts of strontium found in rats nursed not more than four to six hours was 3.01 per cent. The mother had been on the strontium carbonate diet four days. In rats which had been nursed four and nine days, respectively, the mothers having had strontium for two days before the birth of the young, the strontium content amounted to 4.15 and 5.74 per cent, respectively, of the ash of the bodies.

The ash of young rats five days old, whose mothers had had no strontium, contained none of this element. These results are shown in the table given below.

TABLE 6

Rate of deposition of strontium in the bodies of young rats which had secured the strontium through the milk of the mother

NUMBER OF RATS	AGE OF YOUNG	TIME OF FEEDING STRONTIUM TO MOTHER BEFORE YOUNG WERE BORN	TIME OF FEEDING STRONTIUM TO MOTHER AFTER YOUNG WERE BORN	PERCENTAGE OF STRONTIUM IN ASH OF YOUNG
6	5 days		0 days	0
4	4 days	2 days	4 days	4.15
3	9 days	2 days	9 days	5.75
8	4-6 hours	4 days	4 hours	3.01

The data show conclusively that the milk of a mother receiving strontium contains this substance, and the young can receive it through this path.

To determine if strontium can be transmitted through the placenta, pregnant rats receiving rations I and III, and as controls rats receiving ration II, were killed, the young removed from the uteri, the bodies ashed, and analyses made for stron-

tium and calcium. The results are most easily compared from table 7.

The most striking figures in this table are those for the rats whose mothers had received ration I. It is to be observed that the amount of calcium is very low, and the strontium relatively high. The percentage of calcium in the ash of rats whose mothers had received the calcium and strontium diets is apparently close to normal. It will be seen from the figures of the normal rats that the calcium of the ash increases as the gestation period progresses. The data show that strontium can be transmitted through the placenta to the body of the unborn rat.

TABLE 7

Percentage of strontium and calcium found in the bodies of unborn rats whose mothers had received rations I, II, and III

NUMBER OF DAYS PREGNANT MOTHER RE- CEIVED RATION	RATION	NUM- BER OF YOUNG	PER- CENT- AGE CAL- CIUM	PER- CENT- AGE STRON- TIUM	NOTE
10	III, containing stron- tium and calcium	7	10.32	1.91	Pregnant when put on ration
13	I, containing stron- tium	9	3.54	2.34	Pregant when put on ration
21	I, Containing stron- tium	7	3.34	2.88	Entire gestation period
Maintained on ration	II, Strontium-free ration	9	9.9	0	Gestation period about two-thirds completed
Maintained on ration	II, Strontium-free ration	7	13.90	0	Gestation period about completed

From these data it is evident that strontium behaves like calcium in so far as its capability for being deposited in the bones and in the milk is concerned. That the similarity ends there and that strontium can in no way replace calcium physiologically we will endeavor to establish by a description, which is to follow, of the histological pathology of the bones and the physiological behavior of rats fed the rations described above.

To summarize briefly the results of the preceding experiment, we may say that strontium is deposited in the bones of the body rapidly and in considerable quantity. Its entrance is not in-

hibited by the presence of calcium in the diet, but it enters the body of a mature rat more slowly than a young one. It may enter the body through the placenta, the milk of the mother, and the alimentary tract.

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THE ACTION OF CAMPHOR, MENTHOL AND THYMOL ON THE CIRCULATION

REGINALD ST. A. HEATHCOTE

From the Department of Pharmacology, University of Oxford

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LITERATURE

There is a great deal of literature dealing with the action of camphor on the frog heart. This has been well summarised in two papers, that of Plant (23) and that of Joachimoglu (12), and no long summary is needed here. Briefly, there are three main groups into which the previous work may be divided: Heubner (10), Harnack and Witkowski (9), Maki (19), Wiedeman (33), Stockman (29), and Lippens (18) all maintain that camphor acts as a cardiac stimulant; A. Lewin (15) and Schwalb (25) found it to be depressant; Boehme (1), Langaard and Maas (14), and Haemaelaenen (8) considered that it had no action on the normal heart but that, when the heart was previously depressed by chloral, a stimulant action appeared.

There are also some experiments tending to show that differences exist in the action of the three isomers, d-, l- and i-camphor. They are mutually contradictory, no two authors being in agreement, and need not be considered here as the point has not been investigated in this work.

Plant (23) found that camphor was depressant to the normal frog heart but that, in the chloralised frog, the heart was stimulated by both camphor and ether when applied to the exterior of the heart. Joachimoglu (12) repeated these experiments and was unable to confirm them with camphor but with ether in a few cases a positive result was obtained. He found no difference, qualitative or quantitative, to exist between the actions of the isomers. With a concentration which was variable but always

about 1 in 4000 he found that the heart, perfused by Straub's method, showed an augmentation of its beat after a brief depression but there was never any acceleration, usually the reverse. With all other concentrations, both stronger and weaker, the heart was depressed and frequently showed signs of permanent damage in that recovery with Ringer's solution was not complete.

He also investigated the action of menthol. This he found to be purely depressant and to be about ten times as powerful as that of Camphor.

Pellacani (21) has also investigated the action of menthol. He found the frequency of beat to be diminished but the power increased, exactly as was claimed by the earlier workers, e.g., Wiedeman (33), for camphor. Ultimately the heart stopped in diastole.

There do not appear to be any accounts of experiments on the frog heart performed with thymol.

In the mammal, the action of camphor on the peripheral vessels has been investigated by Stockman (29), Winterberg (34), Liebman (also on the pulmonary vessels), (17), and Lichatcheff (16). All of these found that it has a dilator effect. On the other hand, Pilcher and Sollmann (22) found the flow through the kidney, spleen and intestine to be reduced. This was possibly due, as had been suggested by Winterberg who also had observed a diminution of flow through the abdominal vessels, to the lowered systemic blood pressure.

With regard to the heart, A. Lewin (15), after paralysing the vaso-motor center with chloral, obtained a rise of blood pressure on injecting camphor. This he attributed to a direct stimulation of the heart. Seligman (26), Isaak (11), and Plant (23) failed to confirm this but Isaak found that in animals poisoned with phosphorus camphor raised the blood pressure. This also was denied by Plant. Seligman (26) claimed that camphor stimulates the perfused heart. His experiments, however, do not support his view. Gottlieb (3) found, but Winterberg could not corroborate him, that, in a heart lung preparation, in which overdistention had caused the heart to beat feebly, camphor brought about improvement. Winterberg (35) only observed

depression to occur in the heart perfused with camphor. Van Egmond (32) using the myocardiograph and Richards (24) also only found depression to occur on administration of camphor. Lichatcheff (16) confirmed this.

Seligman (26) claimed that fibrillation in the perfused heart, whether arising naturally or through electrical stimulation, could be checked by the addition of camphor to the perfusion fluid. This was confirmed by Gottlieb (3) in the anaesthetised animal. Winterberg (35) repeated the experiments but was unable to corroborate them. Klemperer (13) found that camphor was without action on the naturally fibrillating heart but that it rendered the heart somewhat more resistant to electrical stimulation. Richards (24), on the other hand, found no evidence that camphor could prevent or check fibrillation.

With regard to the action of camphor on the vaso-motor center, the earlier experimenters, e.g., Wiedeman (33), found that, even in curarised animals, camphor caused elevations to appear at irregular intervals on the blood pressure tracing. This they attributed to stimulation of the center. Winterberg (34) found that, after administration of curare in doses sufficient entirely to paralyse the muscles, these elevations no longer appeared. On the other hand, he found that, on injecting amounts of camphor, too small to have any general action, into a carotid artery, there was evidence of a weak and brief stimulation of the center. The elevations of blood pressure, he thought, were to be attributed to reflex stimulation of the center, the irritability of which had been increased by the camphor. Seligman (26) also found that animals which were sufficiently curarised did not show any rise of blood pressure on administration of camphor. Pilcher and Sollmann (22) also found that there might be stimulation of the vaso-motor center by camphor but that it was weak and inconstant with small doses. With doses sufficient to cause convulsions the stimulation might be intense. The action of menthol on the circulation was investigated by Pellacani (21). He found that it caused the appearance of elevations of blood pressure exactly similar to those caused by camphor. His animals were apparently not curarised and so probably the

same explanation may account for them as was advanced by Winterberg for camphor.

Lichatcheff (16) found that menthol was depressant to the perfused rabbit heart and that it dilated the blood vessels.

No experimental work dealing with the action of thymol on the mammal has been found. It is stated by Cushny (2) that it has been known to cause alarming collapse due to its cardiac action, on administration to man.

Camphor is largely in use in most continental countries as a cardiac stimulant especially in moribund cases. Thymol is continually in use in cases of ankylostomiasis. In view of the discrepancies in the accounts of the action of the former and of the lack of experimental evidence concerning the latter, it was thought advisable to endeavour, partially at any rate, to clear up the confusion. Menthol, being closely related chemically to camphor, was added for the sake of comparison.

EXPERIMENTAL

Minimum lethal dose. This has been only roughly determined. A strong (20 to 25 per cent) solution in olive oil was injected under the skin of the flank in rabbits. It was found that the minimum lethal dose per kilogram was, of camphor 2 grams, of menthol 2.6 grams and of thymol 1.5 grams. In all cases, the immediate cause of death was paralysis of the respiratory center.

Frog blood vessels. These were perfused by a modification of the method of Trendelenberg (31). All three drugs exert a weak vaso-constrictor action. The matter is not of great importance and does not need a detailed account. With 1 in 1500 of camphor the rate of flow fell, in seven minutes, from 1.8 cc. per minute to 0.7 cc. Menthol and thymol gave similar results. No dilation was observed with weaker solutions.

Isolated perfused frog heart. The action of these three drugs was determined by the use of a modification of Symes' method (30). It was found to be, in every experiment, purely depressant, both rate and amplitude of the beat being reduced. Prolonged

perfusion usually left some permanent damage in the heart, in that recovery with Ringer's solution was not complete. This has also been observed by Joachimoglu (12). The addition of atropine to the perfusing fluid did not cause any change in the character of the beat, so that the effect is probably caused by a direct action on the cardiac muscle itself. It was found that the greater part of the slowing was due to prolongation of the A-V interval and of the diastolic pause, but there was also some slowing of the actual contraction and relaxation of the muscle. The lowest concentration which would stop the heart in diastole was found to be, of camphor 1 in 1500, of menthol 1 in 10,000, and of thymol 1 in 20,000. To produce any effect at all the weakest solution was, of camphor 1 in 30,000, of menthol 1 in 200,000 and of thymol 1 in 200,000. Tracings to show the action of menthol-thymol are reproduced in figures 1, 2.

Camphor on the chloralised frog heart. The method which has usually been used for this purpose seems exceedingly liable to fallacy. Chloral hydrate was injected in solution into the dorsal lymph sac of the frog and after a sufficient time had elapsed the heart was exposed and any change in its beat recorded when a solution of camphor was dropped on to the exterior.

To obviate this source of error, a method was adopted from that described by Gunn in his paper on "The antagonism between adrenine and chloroform, etc., on the heart" (7). A solution of chloral of a given strength was made up and an exactly similar solution except that, in addition, it contained camphor was prepared. The solution of chloral was first perfused and after it had exerted its full action the solution containing both chloral and camphor was used. One could then be certain that the chloral was not removed from the heart while the camphor was passing through. Solutions of chloral, either 1 in 500 to arrest the heart or 1 in 2000 to depress it only, were used in conjunction with strength of camphor ranging from 1 in 1500 to 1 in 30,000.

If the solution of chloral was strong enough to arrest the heart, further perfusion with camphor was never able to bring about a spontaneous beat. The heart was still irritable as it would give a few beats in response to a mechanical stimulus. Recovery with Ringer's solution was never complete.

The results with weaker solutions of chloral are more useful. It will be seen from figure 3 that, after a short perfusion with chloral, the heart beat became slower and the amplitude less, but it settled down to a fairly constant rate and size. On now

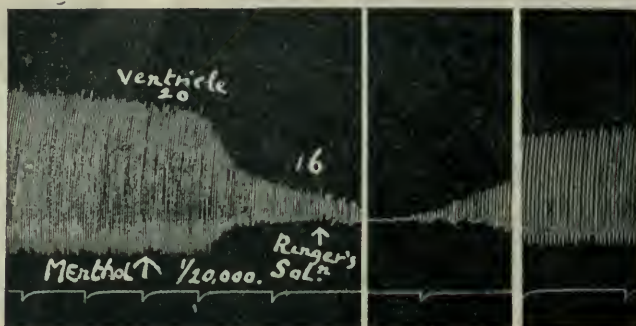


FIG. 1. FROG HEART PERFUSED WITH MENTHOL, $1/20,000$

The beat becomes weaker and slower, ending in arrest in diastole. Recovery with Ringer's solution is nearly complete.

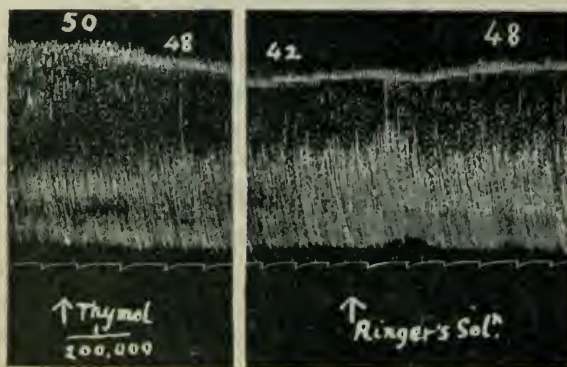


FIG. 2. FROG HEART PERFUSED WITH THYMOL, $1/200,000$

This is about the weakest solution to cause any effect. There is a small decrease in both amplitude and rate. Both recover completely with Ringer's solution.

changing to the mixture of camphor and chloral, the amplitude and rate decreased rapidly, there being a summation of the two actions. That it is a summation can be seen from the next section of the tracing when the solution of chloral only was again

perfused. Although there was only a small increase of rate the amplitude became practically doubled. On changing over to Ringer's solution the heart recovered, almost entirely, its original rate and amplitude. In all these experiments there was never seen any sign of stimulation of the chloralised heart.

Isolated perfused rabbit heart. For these experiments the apparatus of Gunn (5) was used and for the measurement of the coronary output his siphon recorder (6).

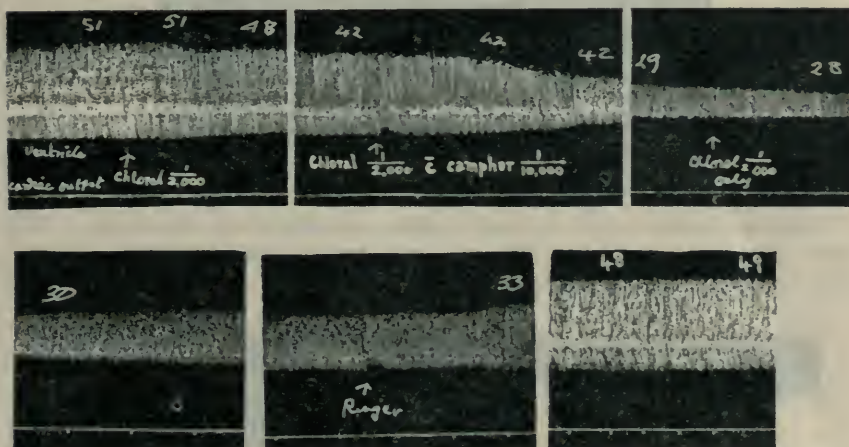


FIG. 3; THIS SHOWS THE ACTION OF CHLORAL, 1/2,000, BOTH WITH AND WITHOUT CAMPHOR 1/10,000

It will be seen that after a certain time of perfusion with chloral the heart-beat is practically constant, both in rate and in size of beat. On perfusion with the mixture, there is a much greater reduction of both, which is very rapid in onset. Perfusion with chloral, again without camphor, brings about a considerable improvement in the amplitude but only a little increase in the rate. Ringer's solution restores the heart to its normal state.

As with the frog so also with the rabbit heart, each of the three drugs has a considerable action on the amplitude of the beat. In contradistinction to the frog, however, the rate was not appreciably affected by any of them. Atropine was unable to produce any modification of the reduction in the size of the beat and presumably the action of the drugs is directly on the heart muscle. The strongest solution of camphor used, 1 in 5000, was not able to arrest the heart in diastole but, with men-

thol 1 in 10,000 and with thymol 1 in 20,000, the heart stopped quickly. Except with very strong solutions of thymol (which sent it into rigor), recovery on perfusion with Locke's solution

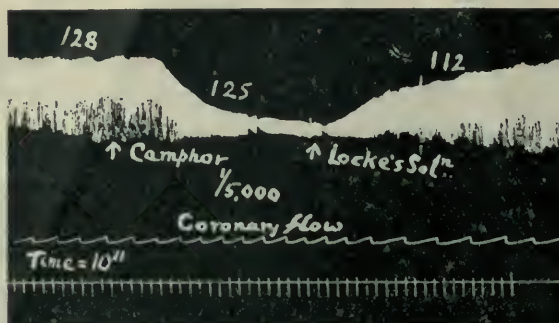


FIG. 4. RABBIT HEART PERFUSED WITH CAMPHOR 1/5,000

The amplitude is rapidly reduced to about one-sixth of the original size. The rate, as usual, is unaffected. The coronary flow is nearly doubled. Locke's solution brings about complete recovery.

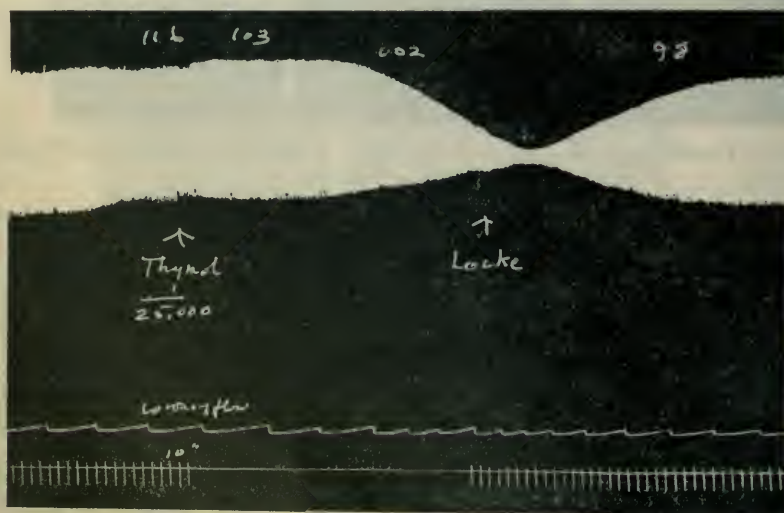


FIG. 5. RABBIT HEART PERFUSED WITH THYMOL 1/25,000

The heart was greatly depressed and would have stopped in diastole had Locke's solution not been turned on. It recovered completely with the latter. The rate was scarcely affected but the coronary flow was more than doubled.

was complete. The weakest solution which had any action was found to be of camphor 1 in 60,000, of menthol 1 in 200,000 and of thymol 1 in 200,000.

The coronary flow was increased by all three drugs, least by camphor and most by thymol.

Figures 4 and 5 show the action of camphor and thymol on the mammalian heart.

Experiments on the circulation

For these experiments cats were employed, either anaesthetised with ether or decerebrated after the method of Sherrington (27). A few cats also were decapitated, also by his method (28). As it has been shown, first by Stockman (29) and later by Morita (20), that the convulsions caused by camphor in mammals have their origin in the cerebral cortex, most of the experiments were done on decerebrate animals and care was taken in those on anaesthetised animals not to administer a convulsive dose.

Preliminary experiments showed that 2 cc. of olive oil could be administered intravenously without causing any appreciable change in the blood pressure. Sometimes, therefore, a strong solution of the drug in oil was given in this manner. On other occasions, the more closely to parallel the usual method of administration in man, a solution was injected under the skin of the flank. Absorption was usually complete within an hour. Intravenous injection of a solution of the drug in Locke's solution had to be given up as, owing to the insolubility of each of them, such large amounts had to be injected that the blood pressure rose to a considerable extent, especially in decapitated animals, and so obscured by the mass of fluid the effects due to the action of the drug.

It was thought that it was possible that subcutaneous injection of camphor might bring about a reflex rise of blood pressure by its irritating action. In view of the experiment on the isolated heart described above, and of those of earlier workers on the peripheral vessels, it did not seem likely that any direct cardiac stimulation would occur.

a. Camphor. The experiments on decapitated animals may be briefly described and dismissed. On subcutaneous injection of camphor, even in large doses, no noticeable change in the blood pressure occurred during an hour. Intravenous injections of camphor in olive oil caused a rapid fall in the blood pressure.

Most of the experiments were done on decerebrated animals. In these, the subcutaneous injection of camphor in olive oil caused a slow fall of blood pressure. In figure 6 is shown a tracing which displays the usual features observed in these experiments. The blood pressure at the commencement was 158 mm. Hg. After the subcutaneous injection of 0.5 gram of camphor per kg. a steady fall of blood pressure commenced and this continued for an hour, at the end of which period the blood pressure had reached 100 mm. This was not the invariable result but the most frequent. The blood pressure was found once to remain constant for an hour and a few times to fall at first and then gradually recover its original height. Never, however, was a rise in blood pressure seen.

With intravenous injections of camphor the fall of blood pressure was much steeper and with doses of about 20 mgm. per kilogram it was very prolonged. A tracing is reproduced in figure 7 to show these results. The blood pressure was 126 mm. Hg and on injection of 4 mgm. of camphor per kilogram it fell to 106 mm. This dose corresponds to the amount usually given subcutaneously in man. From this level it gradually recovered and after twenty-five minutes it had reached practically its original level. Meanwhile 1 cc. of olive oil had been injected and, as was always the case, it had no deleterious effects. Twenty milligrams of camphor per kilogram were now injected which caused the blood pressure to fall steadily to about 80 mm. As there were no signs of recovery in half an hour 40 mgm. per kilogram were injected. There was a marked fall of blood pressure accompanied by some slowing of the heart rate. The blood pressure remained at about 64 mm. until the end of the experiment. A few experiments only were performed on the anaesthetised intact animal. Care was taken not to administer a convulsive dose of the drug. The results were exactly similar to those obtained with the decerebrate preparation and need no further description.

b. *Thymol*. In view of the results obtained with intact and decapitated animals with camphor, it was thought useless to perform this kind of experiment with thymol and menthol.

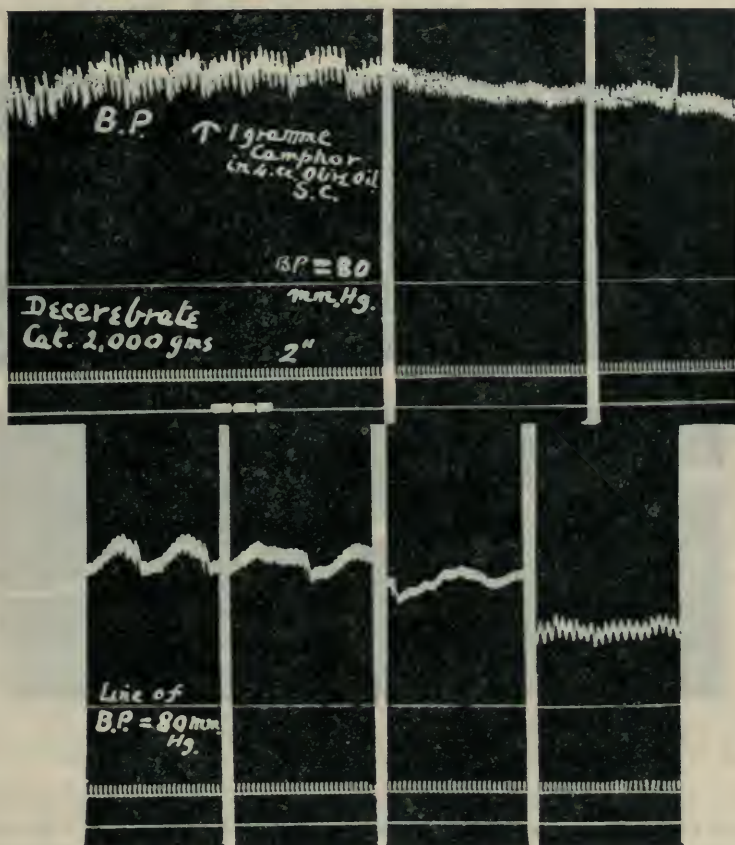


FIG. 6. THIS SHOWS THE ACTION OF CAMPHOR INJECTED SUBCUTANEOUSLY IN A DECEREBRATE CAT

The blood pressure falls steadily during an hour from 158 mm. Hg to about 100. The heart rate is apparently unaffected.

Decerebrated animals alone were employed. With these, the results obtained, with similar dosage of thymol to that used with camphor, were exactly similar to those described above, differing only in degree. With thymol the fall of blood pres-

sure, occurring after both subcutaneous and intravenous injection, was both more rapid and more pronounced. The effects, after intravenous injection, on the heart rate were more marked but with the smaller doses the blood pressure tended to recover more quickly than after camphor.

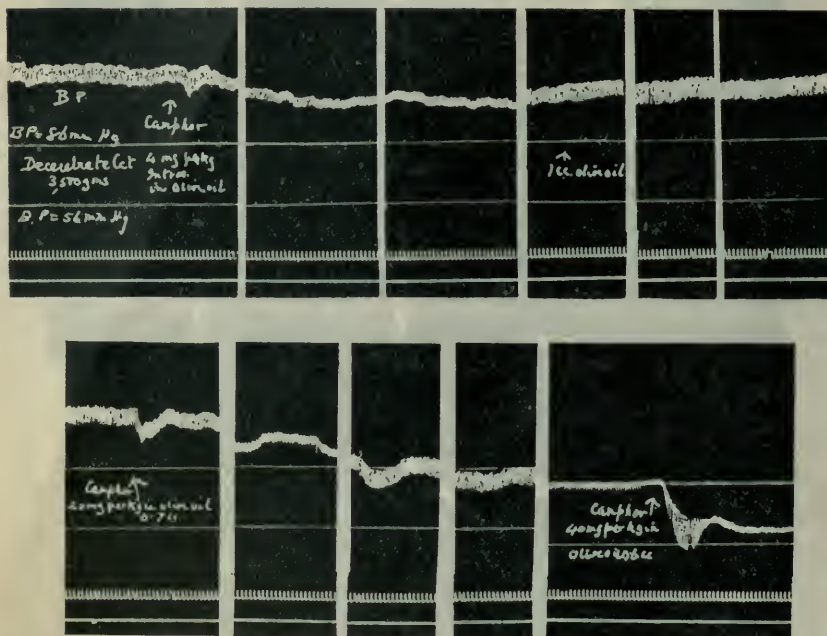


FIG. 7. THIS TRACING SHOWS THE RESULT OF INJECTING CAMPHOR IN SOLUTION IN OLIVE OIL INTRAVENOUSLY IN THE DECEREBRATE CAT

Four milligrams cause a slow fall of blood pressure. After this, there is a gradual return to the former height which is not affected by the injection of olive oil, also intravenously. Twenty milligrams similarly given cause a much more pronounced fall of blood pressure from which there seemed to be no attempt at recovery. Forty milligrams cause a much steeper fall associated with some slowing of the heart. All these doses are calculated per kilogram of cat.

c. Menthol. On subcutaneous injection of amounts of menthol, similar to the doses of camphor employed, in the decerebrate animal no change in the blood pressure could be observed, even after an hour had elapsed. On intravenous injection the fall of blood pressure was smaller and recovery was much more

complete than was found to be the case with either camphor or thymol.

Although it is true that camphor is the most and menthol the least soluble of these drugs, it does not seem probable that the explanation of the differences between their actions can be entirely based on this. Thymol, of intermediate solubility, is the most toxic and has the greatest effect on the blood pressure.

Probably we are here concerned with a double action, on the heart and on the blood vessels. Winterberg claimed and his work has been corroborated by others that camphor is a vaso-dilator. In addition the work detailed above shows that all three drugs are cardiac depressants. It seems most probable that menthol has little or no vaso-dilator action on the systemic vessels and that the fall of blood pressure occurring after intravenous injection is purely cardiac. With the others it is likely that as thymol is far more depressant to the isolated heart than camphor but on intravenous injection, at any rate after the smaller doses, its effects pass off more quickly it has less action on the blood vessels than has camphor.

In all cases it was found that adrenaline would cause its usual large rise of blood pressure when these drugs had been administered and had lowered the blood pressure considerably.

CONCLUSIONS

1. Camphor, thymol and menthol have been shown to depress the isolated heart of both the frog and the rabbit directly, acting on the cardiac muscle.

2. All three dilate the coronary vessels.

3. In anaesthetised animals, camphor, however given, does not cause a rise of blood pressure, if the dose is not sufficient to cause convulsions. Similarly, after the cerebral hemispheres are removed, no rise of blood pressure occurs even with doses up to 1 gram per kilogram.

4. There is no convincing pharmacological evidence that camphor possesses any value whatever as a cardiac or circulatory stimulant. Its value, if any, for this purpose in man should be established or disproved by more exact clinical observations.

5. The unpleasant results which sometimes follow the administration of thymol can be readily explained.

In conclusion the author offers his most grateful thanks to Prof. J. A. Gunn, in whose laboratory and at whose suggestion this work was done. To the British Medical Association he is indebted for the Research Scholarship without the aid of which he could not have attempted it.

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SCIENTIFIC PROCEEDINGS OF THE AMERICAN
SOCIETY FOR PHARMACOLOGY AND
EXPERIMENTAL THERAPEUTICS

FOURTEENTH ANNUAL MEETING, HELD AT TORONTO,
CANADA, DECEMBER 27-28-29, 1922

Edited by the Secretary, E. D. Brown

Quantitative Parallelism of Insulin in Man, Dog and Rabbit. F. H. BANTING (by invitation), C. H. BEST (by invitation), G. M. DOBBIN (by invitation), and J. A. GILCHRIST (by invitation). University of Toronto.

The original preparations of insulin were tested on diabetic dogs previous to the administration of this pancreatic extract to patients suffering from diabetic mellitus. More recently, as has been reported normal rabbits have been extensively used as test objects. Approximately sixty preparations made in the Connaught Antitoxin Laboratories, and a number by the Eli Lilly Company, all of which have been standardized by rabbit tests, have been re-tested on human diabetics in the Hospital of the Department of Soldiers' Civil Re-Establishment. A number of these preparations have also been tested in diabetic dogs in the Department of Pharmacology. The response of the diabetic dog to the subcutaneous injection of insulin varies according to the potency of the extract and with the individuality of the animal. In man it has been found that the drop in blood sugar shows a great variability between man and man on the same dosage. There is, however, a relative constancy in the same man. In the rabbit test the insulin was administered intravenously to a twenty-four hour starved rabbit. There is a considerable variation in rabbits. The area included between the horizontal line drawn through the level of the normal blood sugar in the starved rabbit, and the dip of the curve obtained by plotting the percentage of sugar in the blood over a period of five or six hours gives promise of being a useful index of probable carbohydrate utilization in man.

A Phyto-Pharmacological Study of Some Animal Toxins with Special Reference to a Menotoxin. DAVID I. MACHT AND DOROTHY LUBIN (by invitation). From the Pharmacological Laboratory, Johns Hopkins University.

Macht and Livingston have already shown in connection with a study of cocaine and its derivatives that various drugs affect animal

and plant protoplasm very differently. Thus it was shown that while cocaine is very toxic for animal tissues; it is comparatively little toxic for plant protoplasm. On the other hand, sodium benzoate, which is practically non toxic for animal tissues, was found to be extremely toxic for the roots of *lupinus albus*. These observations suggested the idea that plant cells may be much more sensitive to some animal toxins than animal cells or tissues might be. This was the starting point for the present investigation. Shiek has recently revived or called attention to the ancient popular belief as to the contaminating or deleterious effects of the touch of women at the time of menstruation. He performed a few experiments on cut flowers seemingly corroborating this idea. The present authors decided to investigate this whole question in a more scientific and accurate way by the use of whole living plant organisms, and not cut flowers, inasmuch as the latter method is unreliable, for obvious reasons. The procedure was very much the same as in the study by Macht and Livingston on cocaine and its derivatives.

Seedlings of *lupinus albus* were grown in a perfect nutrient medium (Shive solution) and the rate of growth of the single, straight, well defined roots was measured to within 0.5 mm. Similar seedlings were grown simultaneously and under exactly the same conditions in Shive solution containing a definite amount, (usually 1 per cent), of normal blood serum and on other occasions exactly the same kind of experiments were performed with solutions containing blood serum obtained from menstruating individuals. Whenever possible normal and pathological blood was obtained from the same subjects. The effect of normal serum on the growth of seedlings as compared with normal Shive solution was determined, and in a similar way the effect of menstrual serum obtained from the brachial vein on the growth of seedlings was ascertained. It was found that while the average growth of the seedlings in a 1 per cent solution of normal blood serum in Shive solutions gave about 75 per cent of growth as compared with the normal Shive, a similar solution of menstrual serum affected the seedlings in a much more toxic way. This toxicity expressed itself in two ways. In the first place the absolute growth in the length of the roots was much less, on an average about 50 per cent, as compared with the normal seedlings. In the second place, the rootlets no longer grew in a perfectly straight direction but were curled and distorted in various ways. Numerous experiments repeatedly performed in the above way with numerous samples of sera from normal and menstruating subjects left no doubt as to the greater toxicity of the serum in menstruating subjects as compared with normal ones. The greatest toxicity was usually found at the beginning of menstruation and was demonstrable even during the premenstrual period or a day before the actual onset of the catamenial flow.

Similar experiments were made with samples of saliva obtained from normal and menstruating subjects and a difference in toxicity was conclusively demonstrated in the case of that secretion also. It was

also found that what we may call the "menotoxin" was present likewise in the red blood cells, and some very striking experiments revealed the presence of the same menotoxin in the sweat of menstruating individuals. Experiments are in progress with a view of ascertaining more intimately the chemical nature of the menotoxin. Various glandular extracts such as ovarian, corpus luteum, thyroid, suprarenal, pituitary, etc., have been examined, and more definitely known chemical compounds have also been studied in this connection. Fuller data will be published in due time.

A Contribution to the Pharmacology and Physiology of the Trigonum and the Neck of the Bladder. DAVID I. MACHT AND HUGH H. YOUNG (by invitation). From the Pharmacological Laboratory and the Brady Urological Clinic, Johns Hopkins University.

Embryological, anatomical and surgical studies of the trigonum by Young and Wesson indicate that the trigonum plays an active part in the act of micturition, the opening of the internal sphincter and the closure of the ureteral orifice. In the present investigation pharmacological and physiological experimentation supported by clinical (cystoscopic) observations corroborated this view. The effects of various drugs on the contractions of the bladder reveal different results in the hands of different experimenters; the result obtained with some of the pharmacological agents by certain authors being diametrically the converse of those obtained with the same drugs by other investigators. In the present research, the effect of drugs on bits of excised, surviving tissue from various parts of the bladder were studied. The results obtained were striking, and may be summarized as follows.

The effect of epinephrine on the trigonum vesicae is to produce a powerful *contraction* of muscle excised from this organ. The effect of epinephrine on muscle excised from other parts of the bladder is to produce marked *relaxation* or dilatation. These results have been repeatedly obtained on bladder and trigonum tissue obtained from the following animals; dogs, cats, rats, guinea pigs, rabbits, oxen, horses, sheep and on material obtained from human subjects after surgical operations for resection of the bladder. Both the bladder and the trigonal muscle also react to treatment with ergotoxine.

The effects of pilocarpine, physostigmine, muscarine and atropine on bladder muscle other than from the trigonum is a powerful contraction on treatment with the first three, and a relaxation on treatment with atropine. None of these so-called parasympathetic drugs have any effect on excised surviving trigonal muscle tissue. It is therefore clear, on pharmacological evidence, that while the bladder proper is supplied by nerve endings of myoneural junctions of both the true sympathetic and parasympathetic types, the trigonum is supplied only by the true sympathetic nervous system.

A cystoscopic study of the mechanism of urination was made on a large series of patients. With the cystoscope in position, the bladder was filled to capacity with water and the patient alternately urged to strain (as for voiding urine) and then to relax. While the patient is

straining, a cystoscope can be drawn down into the posterior urethra just as in a tabetic bladder. Anatomical studies show that the circular fibers pass around the bladder until a point is reached just opposite the vesical neck. Here some of the fibers from the region posterior to the vesical orifice swing downward and forward in an oblique direction, passing as thin bands inside the loop of the external longitudinal muscles of the bladder and swinging around the urethra in the region generally opposite the verumontanum where they also form a loop or arch in front of the urethra. This arrangement leaves a short length of the urethra extending anteriorly from the vesical orifice about half way down to the verumontanum, without any investment of fibers arising from the internal circular layer. Other fibers, branching off from this band as it passes downward and forward in its oblique course, extend into the prostate gland. Their course cannot be followed among the prostatic tubules. It seems probable, however, that the prostate represents the posterior portion of this collection of circular fibers extending down around the urethra, which has been invaded and distorted by prostatic tubules.

The vesical orifice, when dilated while making an attempt to void, is not circular but is pear shaped. This shape undoubtedly is due to the *contraction of the trigonal muscle* and the synchronous relaxation of the two loops of muscle about the orifice. The movement is slight and slow on the ventral aspect as the orifice dilates, but on the dorsal side the trigonal muscle accelerates and augments the relaxation of the small loop. The contraction of the trigonal is powerful as shown by the depression of the floor of the urethral orifice and the marked upward movement of the verumontanum.

The part played by the trigon in the opening of the vesical orifice was also studied from the inside of the bladder. Cases of suprapubic cystotomies were used. The cystoscope was passed through the small fistula and the bladder filled with water. Then as the patient strained, the trigon was seen to contract as previously described, the vesical orifice opened, and normal voiding followed.

The above pharmacological and physiological experiments corroborate and amplify the fact already called attention to by one of the authors (Young, vide *Archives of Surgery*, 1921, III, 12.), that the trigonum is a definite entity and that its contractions and relaxation occur independently of those of the rest of the bladder, that the opening of the vesical orifice during urination is not merely a passive inhibitory action, but is primarily the result of the contraction of the powerful trigonal muscle which passes in the form of an arc through the weaker arcuate muscles at the vesical orifice (the so-called vesical "sphincter"), and pulls them open mechanically on contraction. When the trigon is removed micturition is difficult and incomplete; with removal of one-half of the trigon the remaining half functions and the bladder can be entirely emptied. The same is true when the trigon is split. Furthermore the rhythmic tugging of the trigonal muscle at its two upper angles facilitates the opening of the ureters and the flow of the urine from the same.

Concerning the Antiseptic Action of Some Benzyl Compounds. DAVID I. MACHT AND JUSTINA H. HILL (by invitation). From the Pharmacological Laboratory and the Brady Urological Clinic, Johns Hopkins University. Read by title.

Following the discovery of the local antiseptic properties of benzyl alcohol by one of the authors, Macht and Nelson pointed out that this alcohol exhibited also definite antiseptic properties. This was further corroborated in a comparative study of the antiseptic properties of various local anesthetics by Macht, Schwartz, and Satani. These observations stimulated the present investigation which had for its object an examination of various benzyl compounds in respect to their antiseptic and gemicidal properties. The following substances were examined: benzyl alcohol, benzaldehyde, benzyl acetate, benzyl benzoate and a number of natural compounds containing benzyl derivatives such as benzoin, balsam of Peru, balsam of Tolu, oil of cajuput, etc. Inasmuch as most of the above compounds were poorly soluble in water bacteriological studies of the same were made by using solutions of them in oils, so that various degrees of dilution could be obtained. The antiseptic properties were studied on cultures of staphylococcus pyogenes aureus. The results obtained confirmed the previous work on benzyl alcohol and showed that this drug is a powerful antiseptic. Even when diluted with oil 1:100 there was complete sterilization of the cultures after exposure of five hours. Benzaldehyde in dilution of 1:10 was germicidal after exposure of one minute and dilutions in oil of 1:100 inhibited all growth after exposure of three hours. Benzyl acetate and benzyl benzoate also exhibited distinct antiseptic effects and the various galenical preparations such as tincture of benzoin balsam of Peru, etc., also gave a distinct antiseptic effect. The control experiments were made with alcohol and the various oils used, and in this connection another interesting observation was made. On comparing cultures of bacteria mixed with various oils it was found that olive oil itself exhibited a mild degree of antiseptis, a property which was not produced by cottonseed oil, mineral oil peach kernel oil and other oils used.

A quantitative Study of Certain New Local Anesthetics of the Procaine Type. HENRY L. SCHMITZ (by invitation), EDGAR A. RYGH (by invitation) AND A. S. LOEVENHART.

The following compounds have been studied:

1. Cocaine
2. Procaine
 $p\text{-NH}_2\text{C}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{N}(\text{C}_2\text{H}_5)_2\text{HCl}$
3. Para amino benzoyl di-n-propyl amino ethanol hydrochloride
 $p\text{-NH}_2\text{C}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{N}(\text{C}_3\text{H}_7)_2\text{HCl}$
4. Para amino benzoyl di-iso-propyl amino ethanol hydrochloride
 $p\text{-NH}_2\text{C}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{N}(\text{C}_3\text{H}_7)_2\text{HCl}$
5. Para amino benzoyl di-n-butyl amino ethanol hydrochloride
 $p\text{-NH}_2\text{C}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{N}(\text{C}_4\text{H}_9)_2\text{HCl}$
6. Para amino benzoyl di-ethyl-amino propanol hydrochloride
 $p\text{-NH}_2\text{C}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{C}_2\text{H}_5)_2\text{HCl}$

7. Para amino benzoyl di-n-propyl-amino propanol hydrochloride
 $\text{p-NH}_2\text{C}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{C}_3\text{H}_7)_2 \text{HCl}$
8. Para amino benzoyl di-iso-propyl-amino propanol hydrochloride
 $\text{p-NH}_2\text{C}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{C}_3\text{H}_7)_2 \text{HCl}$
9. Para amino benzoyl di-n-butyl-amino propanol hydrochloride
 $\text{p-NH}_2\text{C}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_2\text{N}(\text{C}_4\text{H}_9)_2 \text{HCl}$

These compounds were furnished to us by Dr. Roger Adams, of the University of Illinois, and cocaine and procaine were included as a basis of comparison.

The method used was the intact rabbit cornea and the drugs were instilled into the eye in measured amounts in equal molecular solutions. Most of the work was done at a strength of M/13.6. The material remained in the conjunctival sac one minute and tests were made by means of a simple device for touching the pupil, using exactly the same pressure at each test.¹ The duration of complete anesthesia was thereby determined.

A determination of the toxicity of these drugs on mice was made, the drugs being administered subcutaneously. The minimum lethal dose is the dose that killed two mice out of three within an hour. The results are shown in the following table:

	AVERAGE DURATION OF COMPLETE ANESTHESIA TIME	MINIMUM LETHAL DOSE IN	PRODUCT	THERA- PEUTIC INDEX
	<i>minutes</i>	<i>mgm.</i>		
Cocaine.....	23.0	200	4,600	1.0
Procaine.....	7.5	900	6,750	1.46
N-propyl dimethylene.....	31.0	1,200	37,200	8.0
Iso-propyl dimethylene.....	34.0	1,250	42,500	9.0
N-butyl dimethylene.....	49.0	300	14,700	3.3
Ethyl trimethylene.....	25.5	400	10,200	2.3
N-propyl trimethylene.....	40.0	400	16,000	3.4
Iso-propyl trimethylene.....	42.0	250	10,500	2.3
N-butyl trimethylene.....	52.0	100	5,200	1.2

In the first column is given the average duration of complete anesthesia in a number of animals; in the second column the minimum lethal dose; in the third column is the product of the duration of complete anesthesia times the minimum lethal dose (the larger this product, the greater therapeutic value of the drug); in the fourth column, we have assigned to the product of the duration of anesthesia times the minimum lethal dose (namely 4600), a value of 1, and the fourth column thus gives the therapeutic index of this series of drugs.

¹ In the case of procaine no anesthesia was produced by M/13.6 solution in one minute. Two minutes had to be used to produce any anesthesia. The duration of anesthesia in this case was divided by 2 in order to render it more comparable to the other anesthetics.

It will be noted that the therapeutic index is decidedly the greatest in the n-propyl and iso-propyl derivatives of the dimethylene series. It will be noted that there is a marked increase in the anesthetic power with increase in the molecular weight of the alkyl, both in the dimethylene and trimethylene series. It will also be noted that all the members of the trimethylene series have more powerful local action than the dimethylene, although the difference in the di-butyl derivatives of the two series is not great.

It will also be noted that the di-butyl of the trimethylene series which is on the market under the name of "Butyn," has a therapeutic index lower than any member of the series and is practically as small as that of cocaine.

It will be noted that equimolecular solutions were employed in this work. Had equal percentage solutions been used, the results would have been somewhat different. The butyl derivatives of both series would have shown a less anesthetic power and the therapeutic index would have been even lower than that indicated in the table. The work is to be repeated with equal percentage solutions.

The Relation of Chemical Constitution of Certain Organic Arsenical Compounds to Their Action on the Optic Tract. A. G. YOUNG AND A. S. LOEVENHART. Read by title.

It has been known for some time that Atoxyl and Arsacetin frequently produce amblyopia or blindness in man. Recently, Jacobs and Heidelberger have produced the compound of glycynamide pararsonic acid, called tryparsamide, which has been studied pharmacologically by Brown and Pearce and used clinically in the treatment of trypanosomiasis and syphilis. This substance also produces dimness of vision when used in 5-gram dosage in the human adult. Arsphenamine and neoarsphenamine do not produce this condition.

The object of this study was to find a satisfactory method of determining:

1. Predilection of a compound to the optic tract prior to its clinical use.
2. What structure of these arsenicals determine their ability to cause changes in the optic tract.

On comparing the three products which produce these changes—namely, atoxyl, arsacetin and tryparsamide—it will be seen that they all contain pentavalent arsenic and that they all have an amino or a substitute amino group para to the arsonic acid grouping. They evidently possess in common the property of attacking the retina. Is this property to be attributed to the pentavalent arsenic or to the amino group in para position to the arsenic?

There are four conceivable reasons why neoarsphenamine does not produce these optical changes: (1) because the arsenic is trivalent, (2) because the amino group is in the meta position to the arsenic, (3) because it contains a hydroxyl group ortho to the amino group which may prevent its anchoring in the optic tract, and (4) it may be that the

substances are so toxic otherwise that they cannot be used in quantities sufficient to cause the optical lesions.

We have found that the rabbit is an excellent test object for determining selectivity of an arsenical for the optic tract; that ophthalmoscopic examination furnishes reliable information regarding this property and is confirmable by microscopic examination of the retina.

The sodium salts of the following compounds have been found to cause changes in the optic tract:

Arsacetin or acetyl p-amino phenyl arsonic acid
 Atoxyl or sodium p-arsanilic acid
 3, 4 diamino phenyl arsonic acid
 p-glycine phenyl arsonic acid
 p-glycinamide phenyl arsonic acid (tryparsamide)
 Methyl ureide of phenyl glycinamido p-arsonic acid

The sodium salts of the following compounds do not produce changes in the optic tract:

Sodium arsenite
 Sodium arsenate
 Phenyl arsonic acid
 Meta-arsanilic acid
 3-amino-4-methyl phenyl arsonic acid
 3-phenyl glycinamide, 4-methyl phenyl arsonic acid
 3-amino, 4-hydroxy phenyl arsonic acid
 Arsphenamine
 Neoarsphenamine

Conclusions. The results indicate: (1) that the amino group in the para position to the arsenic is responsible for the injury to the optic tract produced by these compounds, (2) that the amino group in the meta position with respect to the arsenic does not produce changes in the optic tract, (3) that pentavalent arsenic *per se* is not responsible for this property but due to lessened toxicity of the pentavalent compounds, subsequent work may show that these changes can be most readily produced by pentavalent compounds provided the amino group is in the para position.

Drugs and Basal Metabolism. WALTER M. BOOTHBY (by invitation) AND LEONARD G. ROWNTREE. From the Metabolism Laboratory and Division of Medicine, Mayo Foundation, Rochester, Minnesota.

The effect on the basal metabolic rate of the following drugs were studied: acetylsalicylic acid, barbital, benzyl benzoate, caffeine sodium benzoate, cinchophen, citrated caffeine, codeine, corpus luteum, diacetyl-morphine, morphine, ovarian extract, phenobarbital, potassium iodide, pyramidon, radium water, sodium bromide, sodium cacodylate, sodium nitrite, sodium salicylate, solution of hypophysis, sulphonmethane, theobromine sodium salicylate. Neither our experiments nor those of Edsall and Means nor of Means and Higgins are sufficient in number to eliminate the possibility of slight variation in the metabolism by these drugs of the order of 5 per cent. They do however, ex-

clude variations occurring within 90 minutes after administration, comparable in magnitude to the calorigenic action of adrenalin chloride or the specific dynamic action of food.

The Comparative Physiological Activity of Thyroid and Thyroxin.

REID HUNT. From the Department of Pharmacology, Harvard Medical School.

Further work on the acetonitril test for thyroid (the increased resistance of mice to acetonitril after the administration of extremely small amounts of thyroid gland or of "iodothylin" or of "thyroxin") has fully confirmed previous conclusions as to the specificity and delicacy of this test; no iodine compound other than that of the thyroid has been found which gives a reaction at all comparable. Previous work as to the parallelism between the iodine content of thyroid and physiological activity (as determined by the acetonitril test and also by the effects upon growth) has been confirmed; physiologically "inactive" iodine has not been found in any normal thyroid gland.

Administered by mouth in equi-iodine doses thyroxin has invariably proved less active than normal thyroid both by the acetonitril test and by the effects upon growth. That this lower activity of thyroxin is not due to an assumed greater destruction of thyroxin in the intestinal tract is shown by the fact that thyroxin injected intravenously is also less active than thyroid containing an equal amount of iodine.

It is concluded that thyroxin does not fully represent the physiological activity of the thyroid gland when administered as a drug.

Nasal Vasomotor Responses to Changes in Respiration. ARTHUR L. TATUM. University of Chicago.

In a study of vasomotor action by means of the nasal plethysmograph method of Tschalussow we observed that by obstructing the tracheal cannula of dogs anesthetized by barbital, or rabbits by urethane, there occurred a vasoconstriction causing an enlargement of the nasal cavity. Administration of carbon dioxide produced the same results. Artificial respiration so as to produce a resulting period of apnea usually produced a vasodilation.

After division of the cervical sympathetic nerves neither of the above described changes were observed.

We then tried an analogous experiment upon ourselves, using as a criterion the subjective sense of resistance of the nasal chambers to the passage of air during normal breathing. After a fairly prolonged period of voluntary apnea there was felt a distinct diminution of resistance to the passage of air. Conversely after a period of over-ventilation of the lungs a distinct increased resistance of air passage was experienced.

We have asked a considerable number of people to try this experiment on themselves without informing them of results likely to be obtained and so far all who have tried it invariably reported results confirmatory to our own subjective observations.

We cannot explain the subjective sensations on the basis of changes in blood pressure or rate of blood flow incident to differences in intra-thoracic pressure, since the reaction subsequent to voluntary apnea occurs constantly, regardless of whether the apneic period starts at the beginning or at the end of expiration or after forced respiratory effort against the voluntarily closed glottis.

Nerve section in the nasal plethysmograph experiments pretty definitely prove the response to be directly under nerve control, rather than secondary to general circulatory changes.

We believe the volume changes above described constitute a type of adaptive reflex mechanism.

A Method of Studying Peristalsis in Situ. TORALD SOLLMANN.

The walls of the opened abdomen are suspended from a ring so that the abdominal cavity forms a bath for warmed Ringer's solution, in which intestines of the living animal perform very beautifully. Registration by means of levers may thus be supplemented by direct inspection, which is sometimes indispensable for the correct interpretation of the complex peristaltic movements. Drugs may be injected either into the intestinal lumen, or into the circulation.

The results correspond in part to those observed in excised intestine; but the method shows also the effects of the ordinary cathartics, in close correspondence with the clinical phenomena.

Filling the lumen with *normal saline* has relatively little effect, but may somewhat increase peristalsis (fig. 1);¹ but isotonic *magnesium sulphate* produces much more marked stimulation, i.e., increase of tone, force and rate. Hypertonic magnesium sulphate is even more effective (fig. 2). *Castor oil* causes marked increase of peristalsis (fig. 4) and sometimes colic-rings and gas-distention. Soap suds produce intestinal spasm (fig. 5).

Benzyl Benzoate, 1 and 5 per cent into the lumen, unexpectedly always produced marked increase of peristalsis, without any depression (fig. 3). However, the solutions contained 4 per cent and 20 per cent of alcohol, and we have not had time to determine whether this is responsible for the reaction.

Of *autonomic poisons*, *epinephrine*, (fig. 6), intravenously, produced marked relaxation of tonus, whereas the uterus was stimulated (fig. 7).

Pilocarpine, intravenously (fig. 8), and *Physostigmine* and *Barium* (fig. 11) into the lumen (fig. 9) produced intestinal spasm. *Cholin*, into lumen, started active peristalsis (fig. 10). *Atropine* into lumen, tends to relax the pilocarpine contraction (fig. 8). *Peptone-Witte*, intravenously, stimulated the uterus (fig. 12).

Other drugs are still under investigation.

Filtration through Frog-Skin. TORALD SOLLMANN.

The sacs formed by stripping the skin from the hind-legs of frogs are used to study the phenomena of filtration through a relatively

¹ Tracings to illustrate these points were shown at the meeting.

simple living tissue; and for comparing these, with the same tissue in the dying and dead condition. The changes are much more marked in the living than in the dead skin. The rate of filtration (as compared with 0.75 per cent NaCl) is *quickened* by hypertonic solutions, and by isotonic alkali, acid ($N/1000$) and calcium-precipitants, (oxalate, fluoride, citrate). It is *slowed* by distilled water. No definite effect is secured by quinine to 1:1000; and by tannin to 0.5 per cent.

Studies in Protoplasm Poisoning. I. Phenols. L. F. SHACKELL (by invitation). Read by title.

The following conclusions have been drawn from the effects of a series of phenols (carbolic acid, orthocresol, pyrocatechin, resorcinol) on more than 10,000 specimens of the marine isopod, *Limnoria lignorum*:

1. After equilibrium of distribution of a phenol between sea water and an animal immersed in it has been once attained, the poisoning of the animal proceeds with constant velocity. The criterion of toxicity adopted in the first part of this study was the time for initial recovery from paralysis after a given time in the phenol solution. In later work observations were made of the percentages of animals dying after stated periods in a phenol solution. *It was found that poisoning by a given solution proceeded at a uniform rate, which was independent of the criterion adopted.*

2. Since the velocity of reaction is constantly varying during the progress of all chemical changes which conform to the mass law, it would seem that the poisoning of *Limnoria* by a phenol is not a chemical reaction in the ordinary sense. Rather, the poisoning and killing of protoplasm by a phenol may be looked upon as a succession of biophysical phenomena—involving primarily a disturbance of the normal relationships between lipine and proteins; and, secondarily, a progressive aggregation of proteins until irreversible association (coagulation?) occurs. At this point the protoplasm may be said to be dead.

3. When 500 specimens of *Limnoria* were put into 0.5 per cent carbolic acid solution for twenty minutes and then transferred to sea water, there was apparently complete recovery later on; for in twenty-four hours the reactions of these animals were indistinguishable from those of the (500) unpoisoned controls. Nevertheless the mean subsequent duration of life of the poisoned animals was about 30 per cent lower than that of the controls. From this it may be inferred that poisoning catalyzes the normal senescence of protoplasm.

4. Within the limits of the concentrations used (0.125 to 0.5 per cent) the velocity of poisoning by carbolic acid was nearly proportional to the square of the concentration. Five-tenths per cent pyrocatechin was 4.2 times as poisonous as 0.25 per cent; while 0.5 per cent resorcinol was 4.9 times as toxic as 0.25 per cent.

Effect of Morphine and Other Opium Alkaloids on the Muscular Movements of the Alimentary Canal. Preliminary Report. O. H. PLANT AND G. H. MILLER (by invitation). From the Department of Pharmacology, College of Medicine, The State University of Iowa.

The effects on the small intestines were studied on dogs with Thiry-Vella loops of ileum, and the effects on the stomach, in dogs that had permanent gastric fistulae as well as Thiry-Vella loops. After recovery from the operations the contractions of the stomach and of the ileum were recorded, without anaesthesia, by introducing sausage-shaped rubber balloons filled with water through the fistula into the stomach and likewise into the loop and connecting them with Brodie bellows recorders. The doses were injected hypodermically. The action of morphine is typical of this group of alkaloids.

Intestine: Small doses of morphine (0.1 to 0.5 mgm. per kilo body weight, i.e., amounts comparable to therapeutic doses in man) cause marked increase in tone of the intestinal muscles and in the amplitude of the rhythmic contractions. Larger doses produce more striking effect that lasts longer, but is of the same character; even very small amounts (e.g., 0.05 mgm. per kilo produce distinct effect of the same kind. There is no after-effect of relaxation and decrease in contractions, even when the tracing is continued for four or five hours after the injection: the contractions and tone gradually return to normal. The small doses that were used for the most part do not produce purgation. Atropine does not modify this reaction and adrenalin (intravenously) relaxes the gut after morphine as it does normally but the relaxation is not so marked. Tolerance is not developed to this effect of morphine on the intestine: in a dog that had received daily doses of 0.1 mgm. per kilo for thirty-eight days, an injection of 0.05 mgm. per kilo gave the usual result; in another dog that had received daily ascending doses of morphine, at the end of seventy days when the dose was 500 mgm. per day (i.e., 25 mgm. per kilo) the injection of 0.1 mgm. per kilo twelve hours after the last dose still produced distinct increase in tone and in contractions. In this experiment the dog was moderately constipated throughout and did not vomit unless there was a sudden marked increase in the dose.

After five unsuccessful attempts, one Thiry-Vella loop was satisfactorily denervated by cutting the nerve fibers to the loop near the root of its mesentery; in this dog the usual effect of morphine was very much exaggerated, the increase in tone was especially marked, even after very small doses.

Stomach: Small doses of morphine cause marked relaxation of the stomach and decrease in the amplitude of the contractions; the effect lasts as long as does the intestinal reaction, and is not followed by increase in tone or in contractions. When nausea or vomiting occurred the relaxation was not greater than when they were absent. There was never any evidence of a spasmodic contraction of the pyloric portion or antrum, as described by Magnus.

Of the other alkaloids of this group that were tested, Heroine and Codeine have the same sort of effect as Morphine, but codeine is much weaker and heroine apparently somewhat stronger. Papaverine, the only isoquinolin derivative so far tested, has less effect on both stomach and intestine than the others.

If isolated pieces of dogs small intestine are mounted under standard conditions in oxygenated Tyrode's solution, the addition of morphine to the solution in concentrations comparable to those used in intact dogs, always causes relaxation of the segment with decrease in the amplitude of the contractions. The amount of relaxation and lowering of contractions varies in different experiments but this result occurs with the same regularity that the increase in tone and contraction is observed in the intact animals. Other alkaloids of opium have similar effect. We have no adequate explanation as yet for this reversal of the morphine effect in isolated pieces of intestine.

Further Observations on the Carminative Action of Volatile Oils. C. H.

PLANT AND G. H. MILLER (by invitation). From the department of Pharmacology, College of Medicine, The State University of Iowa.

Permanent gastric fistulae and Thiry-Vella loops of ileum were made in dogs and after recovery from the operation, the contractions of stomach and ileum were recorded simultaneously, without anaesthesia, by introducing sausage-shaped rubber ballons filled with water into the stomach through the fistula and into the loop of ileum; records were written on a slow drum by connecting the ballons with Brodie bellows recorders.

When cinnamon water, or aqueous solutions of other carminative oils, was introduced into the stomach through the fistula in amounts varying from 5 to 30 cc., the tone of the stomach was markedly decreased and the amplitude of the rhythmic waves was lessened. The decrease in tone and in the contractions lasted from six to fifteen minutes with gradual return to normal.

When the cinnamon water was introduced in small amounts, 5 to 10 cc., into the loop of ileum, just the opposite occurred, as has already been described by one of us; i.e., tone of the intestinal muscles and amplitude of the contractions are both increased.

In a few experiments there was apparently a reflex relaxation in the loop when the carminative was introduced into the stomach, this result was rarely obtained, however.

The Action of Furfural. HUGH MCGUIGAN.

The following are the results of an investigation of furfural. Details will be published soon.

1. The phenol coefficient of furfural, measured from its bacteriocidal action, is 0.26.

2. A 2 per cent solution of furfural entirely inhibits the action of yeast on dextrose. A 1 per cent solution delays, but does not prevent, this fermentation. Weaker solutions have no effect.

3. Furfural is about one-half as toxic for gold fish as phenol, and about one-third as toxic as formaldehyde for the same animal.

4. Furfural in large doses has a paralytic effect on frogs similar to chloral. There are evidences of stimulation of the central nervous system when smaller doses are used but this is more marked in cats. Locally it is corrosive and anesthetic.

5. Furfural in increasing doses causes unsteady gait, inability to stand and finally paralysis in rabbits. Respiration is at first stimulated and irregular. Cyanosis is marked with larger doses. The reflexes may be increased at first, then decreased with larger doses. The fatal dose by stomach is about 0.8 cc. per kilogram of body weight.

6. The action of furfural on white mice is similar to that on rabbits.

7. When in 5 per cent solution, 0.6 cc. of actual furfural per kilogram of body weight causes only a slight drowsiness in cats; 0.12 cc. of the pure drug per kilogram causes increased irritability, increased respiration, salivation, and finally strychnine-like convulsions.

8. In dogs, the action is much the same as in cats, except that convulsions rarely are elicited. Respiration is paralyzed before the heart stops.

The Efficacy of Arsenic Antidote (Ferri Hydroxidum Cum Magnesii Oxido) U.S.P. HUGH MCGUIGAN, H. V. ATKINSON (by invitation) AND G. A. BROUGH (by invitation).

This investigation was undertaken at the request of the United States Pharmacopoeal Revision Committee. The work is essentially a repetition and confirmation of De Busscher's work. However, we have not found as De Busscher did, that the antidote hastens death when the solid As_2O_3 is used, but in this case we found it, on the average, to delay death a few hours. Neither do we find it of temporary value in case of poisoning by Fowler's Solution, but rather that the antidote is without a significant influence. We feel that the average of De Busscher's work and our own would probably be more nearly correct, that is, that the antidote is without influence on the end result of the action of the arsenic. The delay of death in some cases could be attributed to the colloidal nature of the antidote and not to any specificity of the substance.

In the case of animals which we have marked "survived" we mean that they were alive at the end of two weeks. This time was an arbitrary period taken because of the great variability in the toxicity of the arsenic as pointed out by De Busscher, Schwartze and others who have investigated the subject. The same dose may kill in a few hours, or only after months. This is especially true of the solid As_2O_3 . We have found that those animals which survive two weeks may survive much longer, and by taking two weeks as a limit of observation we do not think there would be any definite change in conclusions had we carried the observations over a longer period. This is especially true since the ratio of treated to untreated animals remains practically the same at this time.

The objection may be raised that we did not use a sufficient quantity of the antidote. The amount we did use did not modify the course of the action or the end result. Also the volume of the official preparation is too great to permit the use of much greater quantities. In the last experiment quantities as high as 375 cc. were used, and again we found that the individual results were of such variance that there was but little difference between the number of deaths of those given antidote and of those without it. In both cases there were marked discrepancies in the results. Again the antidote of the Belgian Pharmacopoeia is much stronger in iron than our own, and De Busscher's work answers this part of the objection. Our own work therefore is in general corroborative of that of De Busscher. The only change produced by the antidote is an unimportant delay in the average time of death when the solid As_2O_3 is used, due, we think, to the nature of the antidote which delays absorption rather than to chemical neutralization of the arsenious trioxide.

Conclusions. 1. Ferri Hydroxidum cum Magnesii Oxido is of no practical value in the treatment of arsenical poisoning. It has no influence in the course of poisoning by Fowler's Solution.

2. There is a slight unimportant delay in the time of death when powdered As_2O_3 is used. This delay seems due to the colloidal nature of the antidote, and to delayed absorption rather than to chemical neutralization.

A detailed account of the experiments, in which we used about 90 dogs and 40 rabbits, will be published in the Journal of the American Pharmaceutical Association.

A Further Study of the Toxicity of Acid Fuchsin with Special Reference to the Relation between Color and Toxicity. J. E. THOMAS (by invitation).

Some years ago (1910) Abel showed that acid fuchsin is a convulsant poison for frogs. In conjunction with his associates Barbour and Macht he later showed, as also did Joseph and Meltzer, that its toxicity is profoundly influenced by a variety of conditions. Such conditions as brain injuries, cardiectomy, fatigue, and deep etherization increase the toxicity of the drug from 2 to 40 times.

The author (1920) presented before this society evidence tending to show that the loss of cerebral inhibition was not an important factor in determining the effect of cerebral injuries in fuchsinized frogs.

The present series of experiments was devised in an effort to find an explanation for this and other peculiarities in the action of this substance. Acid fuchsin is an indicator which gradually changes from a deep magenta red at pH 4.0 to colorless or a faint brownish yellow at about pH 8.5. The greatest rate of change in color is between pH 7.2 and pH 7.6. Decolorization of the dye is associated with changes in the configuration of the molecule. Since color can be gauged accurately by well established methods, it was used as an index of the extent to which molecular rearrangement had progressed in the solutions being studied.

Frogs were perfused through the common aorta with solutions of acid fuchsin having the same concentration but differing in pH, therefore in color. The toxicity of these solutions was judged by the time which elapsed from the beginning of perfusion till tetanus appeared. The more deeply colored solutions were found to be much more toxic.

In these experiments the change in pH alone might account for the difference in toxicity. It happens that weak alkalis decolorize fuchsin solution very slowly so that the same solution will have a different color depending on the time since the alkali was added. A series of frogs perfused with such a solution shows that the toxicity of the drug decreases with the decrease in the intensity of the color, and therefore in the concentration of unchanged fuchsin other conditions remaining constant.

The conclusion is drawn that unchanged fuchsin is more toxic than its nearly colorless alkali salts. This being true, the changes in toxicity of acid fuchsin under the conditions described by various investigators may be due to changes in the pH of the blood and tissues which accompanies fatigue, etherization, and cardiacotomy. Likewise, local acidity at the point of injury may explain the striking results of cerebral and other central nervous system injuries in fuchsinized frogs.

Studies on Some Isomeric and Homologous Fat-Soluble Di-Azo Dyes.

E. W. SCHWARTZE AND J. C. MUNCH (by invitation). Bureau of Chemistry, Washington, D.C.

Experiments of the ordinary type (feeding and intravenous or subcutaneous injections) on fat-soluble dyes are not practicable, especially for acute pharmacological tests. The technical difficulties, however, were overcome by the use of intraperitoneal injections of the dyes in oil solution. As a class, fat-soluble dyes possess an advantage in that they can be prepared in a much purer condition than the water-soluble dyes.

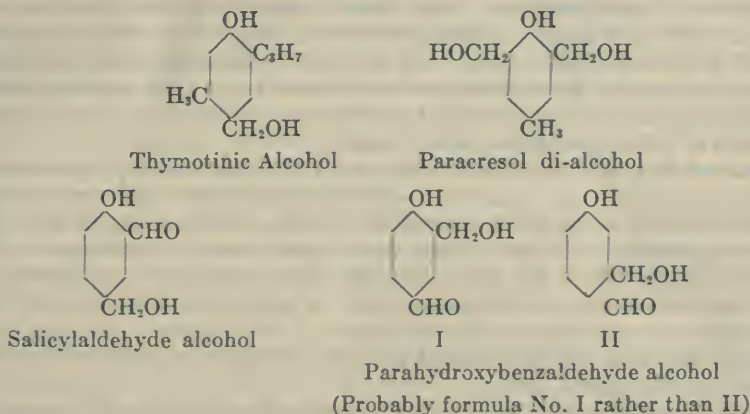
The dyes under study are the benzene and naphthalene diazo derivatives of alpha- and beta-naphthylamine and -naphthol and also of phenol and aniline. The intermediates, as well as the diazotized compounds without the hydroxy or amino groups, are also being studied. It is possible to arrange these compounds in several series, for studying the effects of groups of increasing molecular weight in homologous series, or the effect of internal rearrangement (isomers), and the effect of substitution of the amino for the hydroxy group.

The results of our preliminary experiments with the benzene-diazo-naphthalene series have led us to investigate compounds with similar groupings (homologous series), rather than those with partially similar groupings. The toxicity of alpha- and beta-naphthols and -naphthylamines may for practical purposes be regarded as identical. The addition of the benzene-azo group to these compounds, however, markedly lowers the toxicity in three cases, but in the fourth (diazo (1)-amino (4) derivative of alpha-naphthylamine) it slightly increases it.

These dyes are also being studied in regard to their action on the skin.

The Pharmacological Properties of some Aromatic Alcohols and Aldehyde Alcohols. HERMAN H. JENSEN (by invitation) AND ARTHUR D. HIRSCHFELDER. From the Department of Pharmacology, University of Minnesota.

Four interesting aromatic alcohols, two of them containing aldehyde groups, with the following formulas were synthesized according to the methods given in the literature:¹



It was particularly interesting to compare thymotinic alcohol with thymol. Thymotinic alcohol is practically insoluble in water, soluble in organic solvents. It has a slightly saline, but not a bitter taste. It was injected subcutaneously in 6 and 8 per cent solution in olive oil. Toxicity for frogs is 0.45 to 0.6 mgm. per gram (toxicity of thymol is 0.35 to 0.45 mgm.) causing the same general toxic manifestation prostration, stupor, coma, convulsions and death. In rabbits also the effects of 1.5 grams per kilo in olive oil by stomach tube are the same as those of thymol, prostration, fall of temperature, slowing of heart and respiration, reflexes impaired. These doses are not lethal. The drug has no local anaesthetic action. One 0.01 per cent solutions kill earthworms in one hour, while thymol does this in 0.008 per cent.

Paracresol dialcohol was interesting on account of its two carbinol, one hydroxyl and one methyl group. It is slightly soluble (1 per cent) in water, soluble in alcohol, acetone, ethyl acetate, olive oil, less so in chloroform and ligroin, still less in ether. In frogs it is more stupe-

¹Thymotinic alcohol, Manasse, O., *ibid*, 1894, xxvii, 2409. Paracresol di-alcohol cf. Auwers; Ber., *deutsch. Chem. Gesell*, 1907, xl, 2524. Salicylaldehyde alcohol, Stoermer and Behn, Ber. 1901, xxxiv, 2455. Parahydroxybenzaldehyde (M. P. 133-136°) synthesized according to the same method as the salicylaldehyde compound (M. P. 107-108°) starting with parahydroxybenzaldehyde (M. P. 117°) instead of salicylaldehyde.

ying and less convulsant than the thymotinic alcohol, or than the paracresol from which it is derived. It stupefies rabbits only in lethal doses (1 to 1.5 grams per kilo by mouth.) It has very slight anaesthetic action on the frogs' skin, but no effect on human mucous membrane.

The two aldehyde alcohols are both soluble in about 1 per cent concentration in 0.9 per cent NaCl. The salicylaldehyde compound is bitter and sharply burning, the other alcohol bitter but not burning in taste. Correspondingly this latter compound has less activity, at least this is true of the aldehydes from which it is derived as compared with salicylaldehyde. A 1 per cent solution of the compound has a well marked local anaesthetic action on frogs skin, setting in after one minute and lasting twenty to thirty-five minutes, while compound IV sets in later and lasts half as long.

One-tenth gram per kilo of compound III intravenously gives muscular incoördination without cerebral depression, reflexes normal, pupils dilated, respiration increased in rate, some cyanosis, no effect on temperature. Larger doses sometimes cause convulsions. The lethal dose is 0.3 to 0.6 gram per kilo intravenously. Compound IV has similar effects, about one-fourth to one-third the toxicity, but convulsions set in with smaller fractions of lethal doses. In frogs these convulsions arise in the central nervous system and apparently from action on the spinal cord.

Five-tenths per cent solutions of compound III kills earthworms in one hour but stupefies them in fifteen minutes, while compound IV kills in fifteen hours and stupifies in one hour.

All these substances weaken and slow the exposed frog's heart, and in the rabbit cause a fall of blood pressure and slowing of pulse rate and respiration. The latter fails first.

They all cause relaxation and inhibition of the excised rabbit's intestine.

A Special Feature of the Action of Arsphenamine, Neoarsphenamine and Silver salvarsan. D. E. JACKSON.

In work which has been previously published, it was shown that when solutions of arsphenamine or silver-arsphenamine are injected intravenously into a dog, there is produced a rise in the pulmonary arterial pressure. The extent, and the suddenness of appearance, of this rise depend on the concentration of the solutions and rate of injection, but under ordinary experimental conditions the increase in pulmonary pressure is very great. In extending this work further I have found that neo-arsphenamine is very much less active in causing a rise in pulmonary pressure, and that if the solutions are of moderate concentration and the injections are made at a moderate rate, no increase at all may occur in the pulmonary pressure.

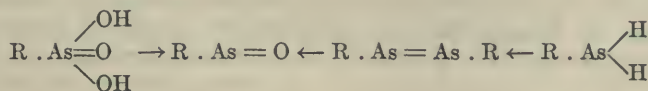
In a study of this problem, the question arose as to how long the increase in pulmonary pressure lasted after it had once been developed to a marked degree. To investigate this point, I have used a modified

mercury manometer in which the rod carrying the float was attached to the lever of a tambour (the bowl of the tambour being removed) in such a manner as to magnify about 8 or 10 times the movements of the mercury column. The anesthetized animals were arranged as usual for recording the carotid pressure and then large injections of the drugs (only arsphenamine and silver arsphenamine) were given in a number of repeated doses by way of the femoral vein. The time of these injections was noted and at given intervals later the chest was opened and the left pulmonary artery was connected to the recording manometer. In this manner it was determined that the abnormal rise in pulmonary pressure was greatly reduced again within fifteen minutes, and that after about one hour it was no longer possible to detect any increase in the pulmonary pressure above what would probably have been found normally in the animal. This normal was determined approximately by experiments on animals which had not been previously given intravenous injections.

It has seemed probable that the length of time required for the pulmonary pressure to return to about its normal level is of significance in relation to the length of duration of the acute symptoms which sometimes develop immediately after intravenous injections in man.

Further Observations on the Relation Between Chemical Constitution and Chemotherapeutic Action of Arsenicals. CARL VOGTLIN, J. M. JOHNSON (by invitation), HELEN DYER (by invitation) AND J. W. THOMPSON (by invitation). Division of Pharmacology, Hygienic Laboratory, United States Public Health Service.

The following compounds were studied: (I) 3-amino-4-hydroxyphenylarsin, (II) 4-sulphonic-phenyl arsonic acid, (III) arsenobenzene-4, 4-disulphonic acid, (IV) 3-amino-4-hydroxyphenyl arsenious sulphide, (V) 3-amino-4-hydroxyphenyl arsenic sulphhydric acid and (VI) 3-amino-4-hydroxyphenylarsenic disulphide. The biological action of these compounds was then compared with that of other arsenicals previously studied. The results substantiate the hypothesis previously advanced, that the "active" modification is represented in each case by the trivalent arsenious oxide:



For 3-amino-4-hydroxyphenyl arsenious oxide ("arsenoxide") it was shown that the lethal concentration for *Trypanosoma equiperdum* in vitro, suspended in blood, is M/10,000, which is the same concentration as that required to kill the parasites in the infected rat (calculated on the basis of total blood volume).

The introduction of the strongly electronegative sulphonic group into the benzene ring, in place of the amphoteric amino phenol group, leads to a very great reduction of both toxicity for the host and parasitocidal power.

There is very little difference in chemotherapeutic action between 3-amino-4-hydroxyphenyl arsenious sulphide and "arsenoxide." If, however, more than one atom of oxygen attached to arsenic is replaced by sulphur (pentavalent di- and trisulphide) a marked modification of biological behavior results, which is expressed by a great increase in toxicity and parasiticide action. This peculiar behavior of the latter compounds is being further investigated.

Viscosity as an Indicator of the Toxicity of Arsphenamine Solutions.

CARL VOEGTLIN, J. M. JOHNSON (by invitation) AND HELEN DYER (by invitation). Division of Pharmacology, Hygienic Laboratory, United States Public Health Service.

The viscosity of arsphenamine solutions (HCl salt) at 20°C. increases rapidly with concentration. When 1 per cent and more dilute solutions stand at constant temperature their viscosity decreases in a regular manner. Solutions of a higher concentration exhibit just the reverse phenomenon, inasmuch as their viscosity increases on standing. These viscosity changes are due to a physical change and not to a change in chemical composition. Given constant temperature, rate of injection and concentration, it was found that the viscosity of such solutions represents a good indicator of their toxicity as determined by intravenous injection into rats of standard size. The toxicity of solutions of high viscosity is several hundred per cent greater than that of solutions of low viscosity. There is a surprising agreement between the toxicity and viscosity of various lots of arsphenamine. Similar observations were also made by using solutions of the monosodium salt of arsphenamine. In this case it is necessary, however, to guard against oxidation of the drug, as this tends to counteract the physical change produced on standing of such solutions. These observations furnish convincing evidence that the so-called toxicity of arsphenamine depends upon two factors: the physical, which causes death by the formation of emboli (acute deaths), and the chemical factor which causes death by the gradual oxidation within the body of the arseno compound to the corresponding highly toxic trivalent arsenoxide (late death).

This research points out the value of measuring the viscosity for the elucidation of the mechanism of the toxic action of colloids. The application of this method to work on the so-called anaphylactoid phenomena produced by a variety of colloids is obvious.

The Effect of Epinephrine Upon the Blood Picture. C. W. EDMUNDS AND RUTH P. STONE (by invitation). University of Michigan.

Epinephrine when administered to dogs in doses of about one milligram per kilo causes a large increase in the number of red blood cells and typical changes in the relative proportions of the white cells. The explanation offered for the increase in red cells is the action of the epinephrine upon the hepatic vein causing constriction, thus increasing the portal pressure and an outpouring of lymph. The polycythemia is not produced in rabbits, due it is said, to the fact that

epinephrine does not affect the liver volume in this animal. The writers studied this reaction in rabbits and found that in the reaction to epinephrine the hepatic vein, the liver volume and the portal blood pressure in rabbits did not differ in any essential detail from that of the dog. Perfusion of rabbit's liver with epinephrine causes constriction (McLaughlin). In dogs contrary to the explanation offered, it was found that elimination of the liver from the circulation by clamping the hepatic artery or coeliac axis and even by the removal of the abdominal organs did not alter the reaction. The administration of egotoxin prevented an increase in red blood cells. The most likely explanation in the opinion of the writers is that the polycythemia is due to the action of the epinephrine upon the bone marrow.

Changes in the Epinephrin Secretion during Cerebral Anemia. J. M. ROGOFF. From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

When the head arteries are occluded, there ensues a rapid and large rise in blood-pressure, beginning with the onset of anemia and continuing throughout the period of occlusion, or until the resulting failure of the bulbar centres causes the blood-pressure to sink to spinal level.

It has been assumed that this vaso-motor reaction to cerebral anemia is intimately associated with epinephrin secretion from the adrenals, although, experimental support for the assumption was lacking. Indeed, recent investigation has afforded proof that this response to occlusion of the head arteries is due to stimulation of the vaso-motor centres and not dependent upon the adrenals.¹

It seemed not unlikely however, that a change in the rate of epinephrin secretion might take place at the time that the vaso-motor centre is responding to the powerful stimulation initiated by the anemia of the bulb, although such a change, if present, would have no important influence upon the blood-pressure reaction.

Stewart and Rogoff² examined the rate of epinephrin output from the adrenals in three cats, fifteen to thirty minutes after permanent cerebral anemia had been induced by ligating the head arteries. While no significant alteration in the rate of epinephrin secretion was demonstrated in these experiments, the possibility of an effect upon the epinephrin-secretory mechanism during the period of profound bulbar disturbance was not studied.

In the present communication are reported the result of eight experiments, on cats, performed with the view of ascertaining the condition of epinephrin output during the period of the anemic blood-pressure response. After obtaining a specimen of adrenal vein blood, before occlusion of the head arteries, other specimens were collected during the occlusion and after release of the cerebral vessels.

¹ Rogoff and Coombs, Amer. Jour. Physiol., 1923.

² Stewart and Rogoff, Amer. Jour. Physiol., 1920, li, 484.

In one experiment the initial rate of epinephrin secretion was 0.00038 mgm. per kilogram per minute (assayed on rabbit intestine segments) and the same output prevailed during the period of cerebral anemia. Occlusion of the head arteries, in this animal, failed to elicit the usual blood-pressure rise. In another cat, the initial rate of epinephrin secretion was 0.00024 mgm. per kilogram per minute. Occlusion of the head arteries was followed by a small rise in blood-pressure, which very soon fell to a very low level. The adrenal blood specimen collected during this period of occlusion had a low concentration of epinephrin, although the rate of flow during the collection of the blood was only 0.14 gram per minute. The output was about one-tenth of the initial rate.

In all of the six other experiments the usual vaso-motor response was obtained on occluding the head arteries. Specimens of adrenal vein blood collected during the early part of the occlusion periods showed definite increases in the rate of epinephrin secretion (raising it to about 3 to 8 times the initial rate) but in only one of these experiments was the rate well above the upper limit of the ordinary range of output. In four out of the six animals the initial rate of secretion was decidedly lower than the usual average. It is not certain whether this may not be associated in some way with the manipulations incident to the isolation of the head arteries. An increase to 6 or 8 times the initial rate, when such rate is already well below the usual average, will of course, bring the increased rate not far from the upper range of the average rate of secretion.

When adrenal vein blood was collected after the release of the head arteries it was observed, in three cases, that the increased rate, prevailing during the period of occlusion, had fallen to about one-half of the augmented secretion in one cat, one-third of the initial rate in another and in the third had returned to the initial rate existing before the cerebral vessels were occluded.

It may be concluded that during the period of vaso-motor response in cerebral anemia the rate of epinephrin secretion from the adrenals may be augmented, but that this has no sensible effect on the character or degree of the responses to the anemia set up in the bulbar centres.

The Action of Pilocarpine upon the Blood Vessels. ERWIN E. NELSON (by invitation) AND GEORGE F. KEIPER, JR. (by invitation). From the Pharmacology Laboratory of the University of Michigan Medical School, Ann Arbor.

The usual teaching with respect to the point of action of pilocarpine is that it stimulates the myoneural junction of the cranio-sacral group of autonomic nerves. A number of cases have recently been described in which there has been stimulation of smooth muscle by pilocarpine, either in the absence of any innervation from the cranio-sacrals, or in the presence of an inhibitory control through this group. On the other hand reports of clear cut inhibition of smooth muscle are practically absent from the literature. For this reason it seemed pertinent

to ask whether the generalization that "pilocarpine stimulates smooth muscle" is not more accurate than that given above, with respect of course only to smooth muscle.

The writers have been investigating the response of the blood vessels as a type of muscle not under the control of the para-sympathetics. Hunt described the vasodilation resulting from the administration of small doses of pilocarpine. The writers have shown that this reaction is local, and that it is reversed by section of the nerves. The dilatation which this section produces (Bayliss) counteracts the effect of depressor drugs acting on the smooth muscle of the arterioles, while it usually intensifies the effect of drugs such as histamine which act on the capillaries (Dale and Richards). Here then is a true case of an inhibitory response of a smooth muscle mechanism to pilocarpine. Neither the usual generalization as to the point of action of pilocarpine nor the alternative suggested above is strengthened by this observation.

The inhibitory type of response is easily converted into motor by the local application of somewhat larger concentrations. Further work is in progress in an attempt at differentiating between a possible nerve end stimulation with small doses as compared to a muscle stimulation with the higher concentrations.

Impurities in Anaesthetic Ether. A. W. ROWE (by invitation). Evans Memorial.

The Pharmacopoeias of the world specify that aldehyde and peroxide are not permissible in anaesthetic ether. Over one-third of the high grade anaesthetic ether on the market today contains appreciable amounts of one or of both of these substances. All anaesthetic ethers should be analyzed as a matter of routine, to preclude the presence of these deleterious materials.

A colorimetric method, based upon fuchsin has been devised for the exact determination of the aldehyde content in anaesthetic ethers. The titrimetric method using cadmium potassium iodide in acid solution and sodium thio-sulphate has been devised for the exact quantitation of peroxide in ether.

Evidence is available showing that the type of container is a factor in the contamination of ether initially pure and that the metal of the container exercises a definite catalytic action on the oxidative reaction producing the impurities.

Effect of Impurities in Anaesthetic Ether. W. L. MENDENHALL. Boston University School of Medicine, and Evans Memorial.

Evidence was presented which indicated that impurities in ether caused injury to lungs, whereby full reflex responses were not secured. Depression of isolated frog hearts by impure ether was noted. It was suggested that lung injury plus circulatory derangement might furnish a basis for post ether pneumonia or bronchitis.

The Action of Aconitine on the Isolated Intestine. WILLIAM SALANT AND LEWIS H. WRIGHT (by invitation). From the Department of Physiology and Pharmacology, University of Georgia. Read by title.

Segments of the small intestine of different animals were suspended in Locke's solution through which a current of air (instead of pure oxygen) was passing constantly, and which was maintained at a uniform temperature. Aconitine introduced into the solution was followed by greatly increased movements, the amplitude being increased several times, while strips of intestine that were inactive at first showed powerful contractions upon the addition of the alkaloid. Tonus was slightly depressed in most experiments but sometimes well marked relaxation of the intestine was observed. A large number of experiments was made with the intestine of the cat and the results were quite uniform. Adrenalin promptly abolished the stimulating effect of aconitine but inhibition by adrenalin was not antagonized by the subsequent use of aconitine. Atropine caused depression after aconitine but complete abolition of movement did not occur. The results in experiments on the intestine of the rabbit were not constant since stimulation as well as depression of the rhythmic contractions occurred, but tonus was always depressed.

A Reversal of the Action of Sodium Citrate on Intestinal Motility and on the Circulation. WILLIAM SALANT AND NATHANIEL KLEITMAN (by invitation). From the Department of Physiology and Pharmacology, University of Georgia. Read by title.

Sodium citrate injected intravenously into different animals produced greatly increased movements of the intestine, stimulated respiration, and caused a fall of blood pressure. The same treatment when preceded by sufficient amounts of atropine had either no effect on the intestine or caused relaxation. Citrate injected after pilocarpine likewise depressed the contractions of the intestine and also caused a considerable rise of blood pressure. In some experiments reversal effects were also obtained when pilocarpine was injected after citrate. This was especially the case with blood pressure which rose considerably when pilocarpine was injected in citrated animals. Particularly striking were the reversal effects obtained with tartrate and pilocarpine. Reversal effects were also observed when sodium citrate and pilocarpine were injected after removal of the adrenals. In fact the rise of blood pressure produced by sodium citrate and pilocarpine was greater than in animals to which these substances were administered when the adrenals were intact.

The Elevation of Temperature Produced by Caffeine in Hypothermia. WILLIAM SALANT AND NATHANIEL KLEITMAN (by invitation). From the Department of Physiology and Pharmacology, University of Georgia. Read by title.

It is currently believed that caffeine raises the temperature in health, but no statements have been found in the literature concerning its

effect when the heat regulating mechanism is disturbed. The present report is the result of studies on the influence of caffeine in dogs when the temperature lowered by means of chloral which was given by mouth. Our experiments show that whereas caffeine exerts very little effect on normal temperature it is otherwise in hypothermia. A fall of temperature was prevented after the administration of chloral when caffeine was injected at the same time or soon after chloral. In another series of experiments caffeine was injected after a fall of temperature of 2 to 4°C. was produced by chloral. The injection of caffeine during this hypothermia was followed by recovery, the temperature becoming stationary after a sufficient amount of caffeine has been absorbed and then gradually rose until it became normal or rose slightly above in one hour and persisted. Observations on the effect of caffeine when given to dogs with normal temperature failed to show any appreciable change in the course of six to seven hours. In a few cases a rise of 0.15 to 0.3°C. occurred but in a number of experiments the temperature remained normal after the administration of caffeine.

A Simple Method for the Analysis of Ether in Air and Its Application to Metabolism Studies. THEO. KRUSE. From the Department of Physiology and Pharmacology, University of Pittsburgh.

In certain physiological studies it is desirable to separate ether from the expired air of anesthetized animals. The principle of the method depends upon the absorption of ether by concentrated sulphuric acid. The absorption occurs rapidly and completely and in no way affects a subsequent analysis of carbon dioxide and oxygen. With the Guthrie (Jour. Biol. Chem., 1921, xlviii, 365) analyzer, it is only necessary to attach two additional absorbers, one containing concentrated sulphuric acid for ether absorption and the other concentrated sulphuric acid or potassium pyrogallate for nitrogen storage. Two or three displacements are adequate for absorption but in practice six or more are used to reduce the temperature error caused by the heat of interaction.

After the utility of the method was established it was found that Horwitz (Diss. 1900 Würzburg) upon the suggestion of Kunkel made use of the same principle. His conclusion regarding slow absorption apparently was a temperature error since the method was tested with very high concentrations of ether, 38 to 84 per cent.

Samples of ether-air mixtures suffer a negligible loss in transfer or analysis when 55 per cent of dessicator calcium chloride solution, acidified with hydrochloric acid is used for displacement in the sampler and analyzer. In the spirometer there is a loss of ether by condensation and solution in water. This is in part preventable by allowing the spirometer bell containing some ether vapor to stand for a day. Stratification in the spirometer is easily corrected before sampling by a hand syringe bulb arrangement by which air is withdrawn and forcibly pumped in again. Fifty to 100 pumps are sufficient to insure adequate mixture. If ether concentrations are to be determined accurately

all rubber connections must be reduced to a minimum. Gum rubber connections should be replaced by white rubber, since the latter takes up very much less vapor.

The method has been successfully applied to study respiratory exchange in anesthetized dogs under various experimental conditions.

Digitalis Standardization. THEO. KRUSE. From the Department of Physiology and Pharmacology, University of Pittsburgh.

The intravenous cat method of Hatcher and Brody (Amer. Jour. Pharm., 1910, lxxii, 360) for the assay of digitalis is generally accepted as superior to some of the older procedures. It differs from them in that absorption is eliminated and therefore indicates maximum activity of a preparation. The chemical method of Knudson and Dresbach (Jour. Pharmacol. 1922, xx, 205) appears to be equally as useful in measuring total activity.

Certain constituents of digitalis are absorbed with difficulty, if at all. When such a constituent is present in excess the cat method fails to give satisfactory information for a suitable estimation of dosage, when the drug is to be administered orally. However, the error is on the safe side because the patient fails to be digitalized.

Such a tincture was submitted to us for standardization, since digitalis effects failed to develop even after the usual dose had been greatly exceeded. It was claimed that by special treatment certain undesirable ingredients had been eliminated and that its strength was 5 times a standard tincture, 50 per cent. The one hour frog method gave the concentration as 3 per cent and the lymph sac contained a residual fluid. Upon this basis the dosage was greatly exceeded on a patient and digitalis effects were subsequently obtained. In view of the fact that the manufacturers claimed 50 per cent by the cat assay method, it was submitted to such a test which revealed that the claimed concentration was essentially correct. Several years later after the Knudson-Dresbach chemical method became known, this same preparation was again retested by the frog method and compared with the chemical method. At this time the result by the frog method was 2 per cent, but the chemical method 30 per cent. It would seem this preparation was poorly absorbed both by the human alimentary tract and the frog lymph sac.

Digitalis preparations intended for oral use certainly should be standardized not only for maximum activity but also for its activity developed after absorption. At present, the frog method seems very suitable for this purpose.

Triple (Magnesium Ammonium) Phosphate Formation and Cystitis from Methyl Alcohol. E. W. SCHWARTZ. Bureau of Chemistry, Washington, D. C.

Retention of urine and feces was observed in the course of feeding experiments in which cats received methyl alcohol in daily doses of 0.5 to 1 cc. Post mortem examination of one cat which succumbed

showed an extremely large quantity of bloody urine in the bladder, from which 2.8 grams of triple phosphate crystals were recovered. The bladder was much thickened. Post mortem examination of other cats (killed) showed a thickened bladder in each. In one, several hundred milligrams of triple phosphate were found. One medium sized grown cat passed 400 cc. of urine at one time. The kidneys of some of the cats were quite enlarged, but did not appear on gross examination to be otherwise markedly abnormal.

A Chemical Method for Assay of Strophanthus Preparations. ARTHUR KNUDSON (by invitation) AND MELVIN DRESBACH (by invitation). From the Laboratories of Biochemistry and Physiology, Albany Medical College, Albany, N. Y.

This work is a continuation of that recently reported before the Society for Experimental Biology and Medicine.¹ This method is based on the same principles as one for digitalis recently published by us.² It is a colorimetric method making use of Baljet's³ reaction, which takes place between the active principles of strophanthus and dilute alkaline picrate solution.

We have tested crystalline Kombe strophanthin, h-strophanthin, g-strophanthin, amorphous strophanthins from various sources, and tinctures and infusions of strophanthus seed from both hispidus and Kombe variety. The chemical method permits of a direct determination of the amounts of strophanthin. The results of the chemical method have been compared with those by the Hatcher and Brody⁴ cat bioassay method.

*Disturbances in the Acid-Base Equilibrium of the Blood by Intravenous Injections.*⁵ P. J. HANZLIK, F. DE EDS (by invitation) AND M. L. TANTER (by invitation). From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco.

The systemic reactions following the intravenous injections of various agents and the responses of foreign protein and non-specific therapy are currently attributed to disturbances in the physical and chemical equilibria of the blood and tissues. A search was made for objective evidences of such disturbances from intravenous injections. Analyses of arterial bloods from 21 different dogs injected with the following hypertonic solutions—10 per cent NaCl, 85 per cent cane-sugar, 50 per cent dextrose, 18 per cent urea, 21 per cent sodium iodide, 50 per cent sodium salicylate and 40 per cent hexamethylenamin and

¹ Knudson, A., and Dresbach, M., *Proceedings Soc. for Exp. Biol. and Med.*, 1922, xix, 389.

² Knudson, A., and Dresbach, M., *Jour. Pharm. and Exp. Therap.*, 1922, xx, 205.

³ Baljet, Henry, *Schweiz. Apoth. Ztg.*, 1918, lvi, 71 and 84.

⁴ Hatcher, R. A., and Brody, J. G., *Amer. Jour. Pharm.*, 1910, lxxxii, 360.

⁵ This investigation was supported in part by a grant from the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association.

also 6 per cent and 25 per cent acacia and 5 per cent gelatin showed the following changes:

1. Marked lowering of pH values (colorimetrically and electrometrically) of the blood occurred, a reduction from pH = 7.3 or 7.2 to pH 6.9 or 6.8 being almost invariably produced. The lowest value observed was pH = 6.6.

2. Simultaneously there was marked reduction in the concentrations of total carbonate and alkali reserve (CO_2 capacity) of the plasma and increased or unchanged concentration of urea, increase in ammonia, increase in lactic acid and dilution of the blood.

3. The reduction in carbonate and alkali reserve appears to be a function of blood dilution, but dilution alone does not explain the lowered pH values, since dilution of the blood and plasma *in vitro* does not alter its reaction. Since the concentration of total CO_2 is reduced, the reduction in pH values is not due to CO_2 but rather to fixed acidosis. In this, lactic acid appears to be concerned in part, at least.

4. Distinct darkening of the blood (reduced hemoglobin) occurred after injection of several neutral compounds, disodium phosphate, acacia and gelatin together with agglutination, hemolysis and rapid sedimentation of corpuscles after acacia and gelatin.

5. Other functions were affected in the usual way, i.e., increases in respiratory and pulse rates, blood pressure, blood volume, occasional rise in rectal temperature of unmorphinized animals, diuresis and tremors, struggling and sometimes convulsions were observed. The animals appeared to succumb more readily to subsequent injections and induced asphyxia.

6. The above changes occurred in morphinized and unmorphinized dogs; with and without artificial respiration. Ether anesthesia tended to interfere with demonstration of the blood changes, because it lowers the pH value and carbonate of the blood.

7. Lowering of pH values of the blood did not occur regularly after injection of 0.9 per cent (pH and carbonate lowered in two out of five experiments) and 0.1 per cent sodium chloride, Tyrode's solution, horse serum, 1.5 per cent and 5 per cent disodium phosphate, 9 per cent bicarbonate and 2 per cent citrate, though dilution and some or no reduction of the carbonate (except after bicarbonate which increased it) of the blood occurred. Lactic acid tended to be augmented, though irregularly, in alkaline bloods after injection of disodium phosphate, bicarbonate and citrate, while ammonia tended to decrease in the same bloods. Agglutination, hemolysis (except with 0.1 per cent NaCl) and sedimentation of corpuscles was not observed with these solutions. Induced asphyxia, which was used as control for the methods of estimating hydrogen ion concentration, invariably lowered the pH values of the blood.

8. The results of the experiments with hypertonic and colloidal solutions demonstrate important departures from the normal in composition of the blood and, therefore, in its functions, and are believed to be of significance in the reactions from intravenous injections.

The Effect upon the Superficial and Deep Temperatures of Certain Substances Applied to the Body Surface. N. B. TAYLOR (by invitation).
From the Physiological Laboratory, University of Toronto.

In the course of our researches upon the spread of heat through animal tissues, some experiments were carried out to determine the normal temperatures of the superficial and deep tissues, we found that the superficial tissues were invariably at a temperature of from 3 to 4 degrees lower than that of the deep tissues, e.g., muscle. This temperature gradient between deep and superficial temperatures could be reversed by the application of heat to the body surface. Recently we have carried out some experiments to determine the effect of certain well known rubefacients upon this temperature gradient.

The temperatures of the subcutaneous tissues and muscles were registered by means of thermocouples encased in hypodermic needles, and inserted to the required depth. The thermocouples were connected in series with a Leeds and Northrup potentiometer and a dead beat galvanometer.

The experiments were carried out upon dogs anesthetized with morphine and ether. A small area in the region of the hip was shaved of hair and the substance applied in a thin layer over the bare skin. The needles containing the thermocouples were inserted beneath this area: the superficial needle immediately beneath the skin, and the deep needle into the muscles of the hip at a depth of about 15 mm. In some cases a third thermocouple was inserted into the rectum to record the general body temperature.

Results. Croton oil. The application of a drop of croton oil spread in a thin film over the surface of the shaved area caused no appreciable rise in the temperature of the subcutaneous tissues as recorded by the superficial needle.

Mustard oil caused no appreciable temperature rise.

Oil of turpentine produced a slight but temporary drop in both the superficial and deep temperatures.

None of these rubefacient substances, when used without a covering of cotton wool, produced any definite rise in temperature of the tissues immediately beneath the area of their application, nor any change in the deeper tissues. On the other hand olive oil and vaseline, if applied in a thick layer, produced a definite temperature rise.

When, after the rubefacient was applied, the shaved area was covered by a small pledget of cotton wool, a very definite rise in temperature resulted. The superficial temperature rose to a point not far removed from the temperature of the deep tissues. In the case of croton oil, the effect of covering the smeared area with cotton wool, was to cause a pronounced and fairly abrupt rise in temperature in the superficial tissues, and a less but quite definite rise, in the temperature of the deep tissues. The depth of the deep needle was 15 mm. and the rise in temperature was about 0.5°C . The rise in temperature of the subcutaneous tissues was 1.4°C . In no case, however, was the temperature gradient obliterated, a slight difference being always apparent

between the deep and superficial temperatures. Similarly, olive oil and vaseline, when combined with cotton wool, produced a pronounced temperature rise in both needles. Cotton wool alone produced a temperature rise equally as great as when the rubefacient was combined with it.

We conclude that these substances have no effect *per se* in raising the temperature of the skin in dogs, but when a rise of temperature does occur, it is due to interference with the radiation of heat from the surface of the skin. For this reason a thick layer of oil and vaseline is more effective than a thin layer, and a layer of cotton wool is more effective than either.

Observations on Substances which Increase the Excitability of the Vomiting Center. SOMA WEISS (by invitation) AND ROBERT A. HATCHER.

The excitability of the vomiting center lying just above the calamus scriptorius, in the floor of the fourth ventricle is increased by the local application of small amounts of many drugs. Emesis was induced in dogs or cats in this way with the following drugs in doses (approximately) expressed in milligrams per kilogram of weight: Apomorphine, 0.0001 mgm.; aconitine 0.0002; morphine, heroine, picrotoxin, 0.001 mgm.; strychnine, brucine, nicotine, epinephrine, histamine, 0.01 mgm.; tyramine 0.1 mgm.

It is certain the amounts just mentioned are greater than those which actually participate in the action on the vomiting center, for reasons which we have stated.

We were unable to induce emesis by the application of atropine, caffeine, codeine, ergotoxine, physostigmine, pilocarpine, quinine, or tartar emetic.

Morphine, in doses somewhat larger than those which increase the excitability of the vomiting center cause depression.

There is a close anatomical relationship of the centers for vomiting and for defecation as shown by the fact that defecation frequently followed the application of drugs, especially picrotoxin, aconitine, and morphine, to the vomiting center.

Both afferent and efferent impulses for defecation and for vomiting pass by way of the vagus and sympathetic.

Much of the older literature relating to the mechanism of vomiting is based upon false hypotheses and is obviously misleading.

Absorption from the Cloaca. W. C. SHARPE (by invitation). Read by title.

A Preliminary Report of Studies on the Bio-Assay of Pituitary Extracts. MAURICE I. SMITH AND WM. T. McCLUSKY (by invitation). Read by title.

The Formation of Liesegangs. HUGH McGUIGAN. Read by title.

Bio-Physical Studies of Effects upon the Temperature of the Brain of Certain Electrolytes. HUGO FRICKE (by invitation) AND B. B. ROSEN (by invitation).

The use of thermocouples for the study of temperature variations in animals organs—as a means of following the heat changes in them—has been described in previous publications. In this report only such changes as are caused by the intravenous injection of two groups of electrolytes will be discussed. The first group includes salts of sodium, calcium and magnesium, a group rich in its theoretical significance since it touches so closely the relation between changes in the oxidative property of blood and variations in its ion concentrations. The second group, comprising the strong inorganic poisons, is well illustrated by sodium cyanide.

Normal electrolytes. An intravenous injection of 2 cc. of a 36 per cent solution of *sodium chloride*—at 37°C.—was made. The injection was followed by a definite rise of about 0.2°C.

The injection of 1 cc. of a 10 per cent solution of CaCl_2 —at 37°C.—produced a decrease of 0.5°C. in the temperature of the brain.

This opposite behavior of the sodium salt to that of calcium is interesting since calcium is usually found to be antagonistic to sodium.

The injection of 1 cc. of a 10 per cent solution of calcium chloride, saturated with sodium chloride, always produced a rise in temperature.

The injection of magnesium sulphate was found to cause a fall of about one degree in the brain temperature. The injection of calcium chloride caused a further drop of one degree. This bears out the statement of Sollmann that the intravenous injection of magnesium lowers the temperature of rabbits; and that the effect is an independent phenomenon, occurring even when narcosis is prevented by calcium.

The injection of sodium chloride before the injection of adrenalin did not inhibit the rise in the temperature of the brain which had previously been demonstrated. This fact becomes more striking when contrasted with the effect of the injection of adrenalin after the injection of magnesium sulphate when only a small increase in the brain temperature is produced.

We may conclude, therefore, that the injection of sodium chloride, of calcium chloride, and of magnesium sulphate alters the degree of oxidation within the brain cells, that is, sodium increases and calcium and magnesium decrease oxidation.

Electrolyte poisons. The effect upon the oxidative power of the brain of a strong poison which is also an inorganic salt is well illustrated by observations of the temperature changes in the brain after the injection of sodium cyanide. Doses varying from 1 cc. of 0.001 N to 0.3 cc. of 0.3 N of sodium cyanide solutions were injected intravenously. After the injection of 0.001 N solution a slight rise in the temperature of the brain was usually noted. In a few instances a slight decrease in brain temperature followed the injection, the rabbits in these cases probably being hyper-sensitive to the poison. The injection of 1 cc. of 0.01 N solution caused a drop in the temperature of the brain while

an injection of 0.7 cc. of 0.1 N solution caused a decrease in temperature followed by a rise during which violent convulsions occurred. All of the above doses were below the maximum fatal dose; the injection of a still stronger dose—0.3 cc. of 0.3 N solution—sufficient to produce immediate death—caused an immediate decrease of the brain temperature, followed quickly by death.

Sollmann states that "injection of cyanides, in doses small enough to allow the action to be watched, is seen to consist of a fleeting stimulation of certain parts of the central nervous system, followed by depression and paralysis." The temperature changes observed by us illustrate this stimulation for the weak doses and depression for the stronger. The effects observed are undoubtedly due to oxidation changes in the brain, for the injection of cyanides are found to greatly increase the blood circulation, intensely stimulating the vasomotor center, with a corresponding rise in blood pressure. If the observed changes in temperature were due chiefly to changes in the circulation of the blood then the results obtained would be opposite to those we have observed.

Summary. 1. The antagonistic relations, common to certain ions, such as sodium and calcium, are illustrated by a study of these effects upon the temperature of the brain.

2. The injection of minute doses of a strong inorganic poison—sodium cyanide—was followed by an increase in the temperature of the brain; stronger doses caused the temperature of the brain to fall.

Bio-Physical Studies of the Effects of Various Drugs upon the Temperature of the Brain and the Liver. I. Strychnine; II. Morphine; III. Bromides; IV. Curare; V. Atropine; VI. Caffeine; VII. Alcohol. GEORGE W. CRILE, AMY F. ROWLAND (by invitation) AND S. W. WALLACE (by invitation).

In previous reports of thermo-electric studies of temperature variations in animal tissues we have noted that the injection of adrenalin in normal animals was uniformly followed by a rise in temperature of about 0.5°C., the periods of rise and fall being practically uniform, the curve being completed in approximately ten minutes. If we are correct in our interpretation that variations in the temperature of the brain indicate alterations in its oxidative power, then this response to adrenalin is an indication of the oxidative power, then this response to adrenalin is an indication of the oxidative power of the brain and it should be possible to compare the effects of various agents upon the oxidative power of the brain by measuring the response of the brain to the injection of adrenalin after the injection or application of those agents. In the following studies the effects of various drugs upon the oxidative power of the brain and also of the liver have been observed the variations in temperature being measured by means of specially constructed thermocouples.

Our findings may be summarized briefly as follows:

Strychnine: The injection of adrenalin in the presence of strychnine produces not only a characteristic rise in the temperature of the brain but also a marked decrease in the temperature of the liver.

Morphine: When adrenalin is injected in the presence of morphine, the temperature response of the brain is diminished in direct relation to the depth of narcosis.

Bromides: The injection of adrenalin in animals which had received heavy doses of bromides for three consecutive days produced no notable variation from the normal response.

Curare: It appeared that it might be of interest to establish the oxidative function of the brain in the absence of any metabolic activity in the voluntary muscular system. Since curare acts specifically on the neuro-muscular plates its injection would cut down the metabolism of the muscular system as a whole. In two animals therefore 10 mgm. of curare in a 0.5 per cent solution was injected intramuscularly. Artificial respiration was established and the usual injection of adrenalin was given. The only alteration in the response of the brain was that the temperature rise was somewhat delayed and was less than the normal average response but not less than the response observed in some normal animals. There was no effect upon the temperature of the liver.

Atropine: The injection of adrenalin after the injection of atropine produced no variation from the normal response of the brain to the adrenalin. However, a greater rise in the temperature of the liver was noted.

Caffeine: Injection of adrenalin in animals which had received preliminary subcutaneous doses of caffeine, was followed by a rise in the temperature of the brain, which did not vary in amount from that observed in normal animals but did vary from the normal response. The temperature rose to the maximum point with extreme rapidity.

Alcohol: Alcohol of itself alone produces a fall in temperature corresponding to that observed in rabbits in shock; while the injection of adrenalin in these animals produces a characteristic rise in temperature this rise is followed by a fall which exceeds that observed in normal rabbits.

THE INTRA-HEPATIC ADMINISTRATION OF DRUGS

J. A. WADDELL

From the Pharmacological Laboratory of the University of Virginia

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From a consideration of its abundant blood supply and its numerous lymph channels, one would deduce that small volumes of solutions injected under the capsule or into the parenchyma of the liver would be rapidly absorbed into the general circulation. An examination of literature, however, has failed to disclose any references to this organ having been employed as a route of administration of drugs for either clinical or experimental purposes, though investigations have been made of the injection of substances into the parenchyma for the study of the local phenomena of injury and repair (Lapeyre (1) and Auché and Couturier (2)) and for curative effects in certain diseased conditions of the liver itself (John (3)). In none of these were the observers concerned with systemic effects arising from a possible absorption of the drug into the general circulation.

For several years, in my laboratory, intra-hepatic administration has been employed in the case of various small laboratory animals such as frogs, turtles, and rats. It was determined that various substances administered by this route are rapidly absorbed into the blood stream and exhibit their effects almost as quickly as after intravenous injection (4). Their physiological effects were elicited with greater uniformity as to dosage and time interval than could be obtained by direct application to the organ or tissue ("dropping the solution on") or by subcutaneous or gastro-intestinal administration.

Among a number of other drugs, I have administered by this route particularly strychnine, caffeine, pilocarpine, atropine, quinidine, digitalis principles, strophantin, helleborein, alcohol, chloroform, and synthetic dyestuffs. The method has proved a

great time saver in the study of effects on the heart, salivary glands, gastro-intestinal tract, and central nervous system.

The procedure consists in exposing a portion of a lobe of the liver and injecting a solution of the drug in question sub-peritoneally or at a depth of 1 or 2 mm. into the parenchyma. The latter gives better results in the case of less potent agents like quinidine and caffeine, while the former is equally effective with very potent ones such as strychnine and strophantin. The solution should be reasonably concentrated so that the volume of fluid containing an effective dose need not be large. Usually this should not be more than 0.1 to 0.2 ml., though in some instances as much as 1 ml. has been injected. These large volumes must obviously inflict severe trauma. Where small quantities only are used, repeated injections, as for a study of the effects and antagonisms of pilocarpine and atropine, may be made into the same lobe, though if the individual injection has been large it would be preferable to employ another lobe.

A few tracings and protocols in brief form showing the rapidity of absorption and resulting effects are as follows:

1. *Protocol.* Frog 30 grams brain pithed; right lobe of liver exposed and strychnine, 0.05 cc. of 0.1 per cent injected. Increased reflexes were shown within one minute and tetanus within two. Chloroform was then immediately administered by the same route, the spasms ceasing one minute later and cardiac death following within another minute.

2. *Tracing.* Heart of turtle, showing pilocarpine action within five seconds and atropine antagonism within ten. The long latent period in the case of the latter drug as compared with the former is no doubt due to the impairment of the circulation (slowing of heart rate).

3. *Protocol.* Wild rat; 200 grams; urethane, 0.75 cc. of 10 per cent per rectum. After complete anaesthesia, the abdomen was opened and arranged for observing the intestines and the following drugs were injected into the right lobe: (a) pilocarpine, 0.13 cc. of 1 per cent producing active increase in peristalsis and profuse flow of saliva within one minute; (b) atropine, 0.13 cc. of 0.1 per cent, antagonizing the pilocarpine within half a minute; (c) strychnine, 0.4 cc. of 0.2 per cent, producing increased reflexes and convulsive respiration in two minutes and tetanus in four minutes; (d) chloroform, followed by death in two minutes.

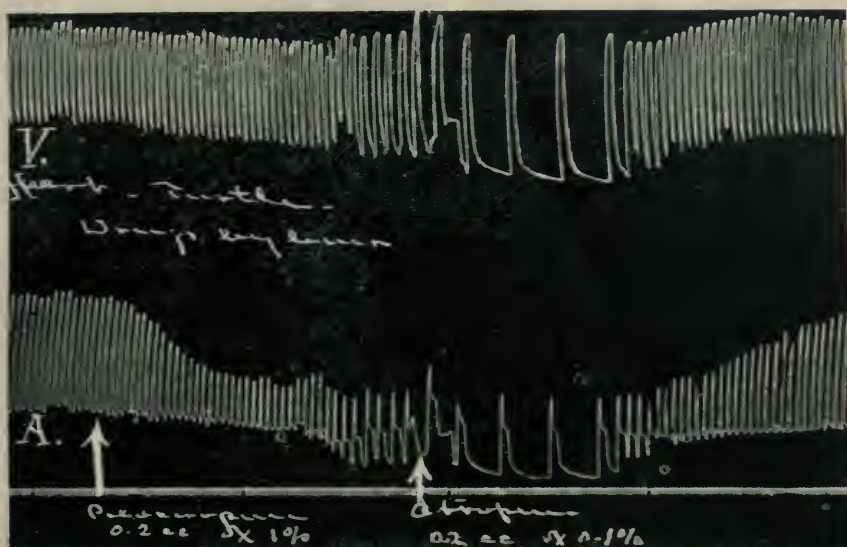


FIG. 1

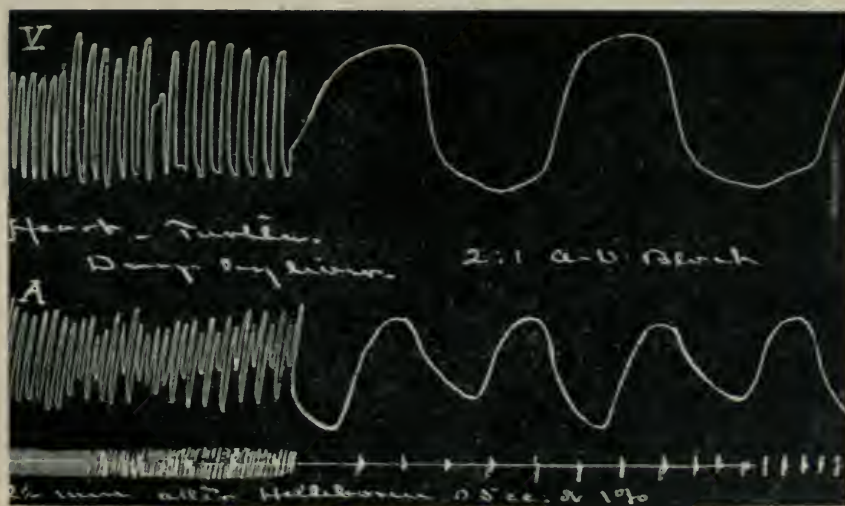


FIG. 2

4. *Tracing.* Heart of turtle, exhibiting auriculo-ventricular block at two and one-half minutes after helleborein per liver. The rate of the kymograph was quickened at the end to aid in analyzing the change in the sequence of the chambers.

In experimental work this method of intra-hepatic administration has certain advantages over some of those commonly employed, as follows:

1. In definiteness of dosage, it is superior to direct application and gastro-intestinal administration, and approximates even the intravenous.

2. In quickness of response (rapidity of absorption), it surpasses the subcutaneous, gastro-intestinal, and other routes, except the intravenous.

3. In time saved in preparing small animals, it is a decided gain over the intravenous.

I have in the process of completion further work bearing on the route of absorption (venous or lymphatic), the rate of absorption, and the quantitative effects by this as compared with other routes of administration; and, in addition, its applicability in survival experiments.

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DOES THE REACTION TO ADRENALIN OBEY WEBER'S LAW?

D. MURRAY LYON

From the Department of Pharmacology, University of Edinburgh

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It has frequently been noted in pharmacological work, that when increasing quantities of a substance are employed, the responses to the higher doses fall short of expectation. This relationship between cause and effect although not a simple ratio, is probably not a haphazard affair, but must follow some definite law. The apparent shortage found in such experiments recalls the case of the special senses where, as the stimuli are increased arithmetically, the effects only increase logarithmically (Weber's Law).

The same failure in response to higher doses was remarked in the case of adrenalin by Hunt (1), who shewed that whereas 0.000083 mgm. per kilogram body weight produced a rise of 5 mm. Hg in the blood pressure, 0.005 mgm. only raised the pressure 66 mm. Varying doses produce varying results, but Baylac (2) found that in animals the same dose of adrenalin repeated many times always produced the same effect. Elliot (3), who re-examined the question, suggested that after frequent repetition of the drug the threshold of stimulation might be raised. The following experiments were undertaken in order to see whether Weber's Law was in operation in the case of the pressor reaction to adrenalin. The case seemed a favorable one for testing whether this Law could be applied outside the field of psychology, for the drug acts quickly, its effects can be readily recorded and most of the factors can be controlled.

As subjects, cats were selected. The animals were anesthetized with ether, decerebrated in order to cut off any impulses from the higher centers, and artificial respiration was continued with the Brodie pump. The records of blood pressure changes were

taken from the carotid artery. The nerves in both carotid sheaths were divided and both femoral veins were exposed to facilitate administration of the drug. Adrenalin chloride (Parke, Davis & Co.) was employed in these experiments, the actual strength of the fluid being chosen so as to give a blood pressure reaction of suitable size. Dilutions of from 1:1000 to 1:20,000 have been used, and of the solution chosen, a series of intravenous injections was given, the doses ranging from 0.5 to 1 ccm.

Adrenalin is believed to act quantitatively, being used up as it produces its effect in the tissues (4, 5). The effect recorded (blood pressure readings) should therefore indicate how much of the substance is being destroyed at any given moment, and the rate of this destruction is doubtless proportional to the concentration of adrenalin in the circulating blood (Mass Action Law). The intravenous injection of adrenalin solution is followed after a short interval by a rapid rise in the blood pressure, the height reached and the duration of the reaction depending on the amount of the drug given. During the latent period the injected material is passing from the femoral vein through the heart to the arterial system. As the adrenalin begins to act on the arteries its effect appears and the pressure rises rapidly until all the drug is in the circulation. At this moment the maximum increase is evident. The constrictor action of adrenalin is short-lived and, as the material is used up, the blood pressure falls. During the short first phase of the decline the curve is convex upwards. In the second phase the curve becomes concave upwards, the rate of the fall increasing for a time and then becoming ever more slowed as the pressure approaches the original level. The second phase of the blood pressure decline resembles the familiar exponential "die away" curve. In the earlier records the curves were frequently deformed by a secondary depression which might appear on the rise or the fall or might obliterate the apex altogether. This depression no longer occurred in those cases where the vagi were previously cut.

Some difficulty has been experienced in measuring the records. To gauge the effect of a dose it would seem best to measure the height of the rise of pressure above the level of blood pressure

in the resting state immediately preceding the injection. It was soon found that, as the experiment advanced, the resting level of the blood pressure tended gradually to sink to a lower plane, and this led to alterations in the degree of response (*vide infra*).

Better results could be obtained by measuring the maxima from the average resting level, or, what amounts to the same thing, from zero. In several cases both procedures have been employed and the results are similar, though a closer agreement is given by the second method. To make a strict comparison between the reactions to various doses it would be necessary to have the resting level of the blood pressure uniform between each observation, but although this ideal has not been attainable, except over very short periods, the results are sufficiently accurate for our purpose.

A number of animals have been examined and in each several series of observations have been made. All the results point in the same direction. It will be sufficient to study one set of records in detail. When the data are plotted out so that the abscissa shows dosage and the ordinates pressure in millimeters of mercury, the maximum points of blood pressure will be found to lie in a curve which rises steeply at first and then flattens out (fig. 1). This type of curve suggests a logarithmic relationship. If it is assumed that the Weber-Fechner Law holds good, the formula expressing this relationship will be—"The effect is proportional to the natural logarithm of the stimulus," or $E = N \log_e S + C$, where E = effect, i.e., blood pressure rise, N = a constant which expresses the sensitiveness of the reacting mechanism, S = the amount of adrenalin employed, and C is an added constant which will not alter the form of the curve but merely controls its distance above zero.

In figure 1 are plotted out the maxima of a series of curves which have been measured from zero and not from the resting level of the blood pressure. The resting level of blood pressure present immediately before the injection is also shown in the chart below each maximum reading. The continuous line *A* has been obtained from the observed figures by the method of least squares and corresponds to the formula $E = 21.43 \log_e S + 91.83$.

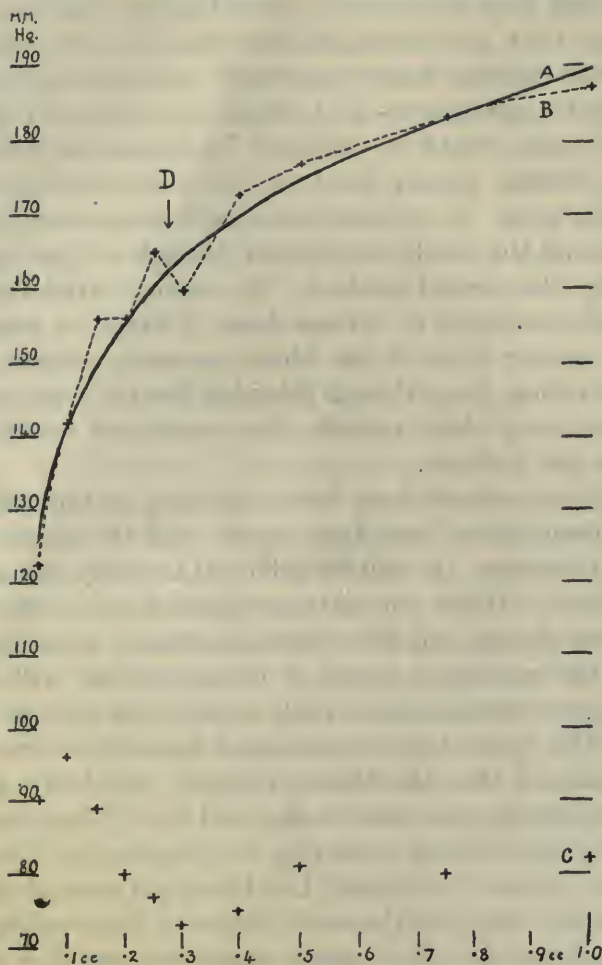


FIG. 1

Ordinates give blood pressure in millimeters of mercury, the abscissae, dosage in fractions of a cubic centimeter of a 1:10,000 solution of adrenalin chloride. Line A is the logarithmic curve calculated from the formula $E = 21.43 \log_e S + 91.83$. The points on line B are the observed maxima of a series of blood pressure rises. At the foot of the figure under each maximum point, is shown the level from which the reaction started. At point D a clot had to be removed from the cannula in the carotid.



FIG. 2. BLOOD PRESSURE TRACING

Showing that the response to a uniform dose of adrenalin chloride varies with the height at which the blood pressure stands when the drug comes into action. Curve *A* followed 1 cc. 1:1000 solution; rises *B*, *C*, *D*, *E*, *F*, *G* all followed doses of 0.2 cc. 1:10,000 adrenalin.

The observed and the calculated figures coincide so closely that it may be said that the blood pressure response to adrenalin obeys Weber's Law. This is an extremely important fact for it infers that the drug does not produce uniform results under varying conditions. For example, the increase of the dose from 0.1 to 0.2 cc. causes an extra rise of 15 mm. Hg (141 to 156) whereas an increase of similar magnitude (0.1 cc.) between 0.9 to 1 cc. raises the pressure only 3 points (187 to 190), and to cause an increase of 15 points at this level (187) would require a dose of adrenalin very much greater than that which suffices to produce the same increase at 141 mm. Hg.

This varying value has been illustrated in another way. A large dose of adrenalin (1 cc. 1:1000 solution) was given in order to raise the blood pressure to a considerable height and produce a slowly diminishing curve. At various points on this falling curve small doses (0.2 cc. 1:10,000 solution) were injected. These produced a series of secondary waves (fig. 2). At a glance it will be seen that the responses to a uniform dose depend on the level at which they commence. The accompanying table gives the actual measurements. During the latent period the main curve continues to fall, then at a definite point the fall gives place to a rise. At this point it might be confidently assumed that the newly added adrenalin has come into action. This point has been taken as the base in the measurements rather than the level actually corresponding to the time of injection. The height of the wave of disturbance caused by the small dose of adrenalin is measured from this point to the maximum of the secondary curve. It should be noticed, however, that as the base line (the large curve) is constantly falling the true magnitude of the disturbance should be somewhat greater. In the table no account of this has been taken because of the difficulty of determining the exact position of the main curve at the point corresponding to the maximum of the secondary one.

TABLE 1

HEIGHT	MAIN CURVE	SECONDARY CURVES					
Beginning.....	94	246	217	197	180	160	136
Maximum.....	260	264	248	239	227	215	199
Wave.....	166	18	31	42	47	55	63
Dose.....	1 cc. 1:1000	0.2 cc. 1:10,000					

SUMMARY

1. Repeated doses of a uniform amount of adrenalin chloride solution produce the same increase in blood pressure only if the resting level of the pressure immediately preceding the injection is the same in each case.

2. When the resting levels differ, the blood pressure responses to uniform doses of adrenalin vary, the magnitude of the disturbance diminishing as the resting level rises. In other words, a much larger dose is required to produce the same effect at a higher level.

3. When the amount of adrenalin administered is increased arithmetically, the resultant blood pressure rises do not follow suit but bear a logarithmic relationship to the stimulus, obeying the Weber-Fechner Law.

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THE TOXICITY OF CARBON TETRACHLORIDE: IN RELATION TO LIVER FUNCTION AS TESTED BY PHENOLTETRACHLOROPHTHALEIN

PAUL D. LAMSON AND A. J. McLEAN

From the Pharmacological Laboratory, Johns Hopkins University

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The use of carbon tetrachloride as an efficient anthelmintic, was advocated by Hall (3) in 1921 after experiments on dogs; he subsequently experimented on monkeys and himself (4), concluding that 3 cc. of the drug (0.05 cc./kilo) could be taken by an adult human being without untoward effects; this is the average dose used clinically at the present time. Smillie and Pessoa (14) have found the drug remarkably efficient in removing *Necator americanus*, 98 per cent of the parasites being removed from human beings after administration of a single 3 cc. dose. The drug has been extensively experimented with in field work, under proper medical supervision, and there also has been shown unusually efficient (6, 15).

Escobar (2) and Nicholls and Hampton (8) have given large doses to human beings without observing ill effects, Escobar administering as high as 50 cc. to an individual without observing effects other than dizziness and sleepiness, while Nicholls and Hampton found at autopsy no necrosis of the liver after administration four days previously of 12 cc. of the drug to a condemned criminal. Hall, furthermore, has given as high as 300 cc. in a single dose to dogs, without causing evident toxic symptoms (5, postscript). Lake (5) has investigated the effect upon monkeys, giving divided doses over a period of twelve days, up to a total of 6 cc./kilo,—over 100 times the dose indicated for man—and found at autopsy “no changes which could be ascribed to carbon tetrachloride.”

Isolated cases of intoxication by the drug, however, have been reported in human beings, these usually occurring in chronic alcoholics, and after a lapse of thirty-six to forty-eight hours (6, 7, 14). Lambert (6) reports but three deaths in the course of 50,000 treatments, two of these being in children under eight years of age, the deaths occurring on the second and fourth days after administration; on account of the extremely small dose of carbon tetrachloride administered and the "proliferative changes about the bile ducts," the possibility that carbon tetrachloride was not the sole cause of death might be considered. Pessoa and Meyer (9) have found also in dogs that doses from 0.3 to 0.5 gram/kilo produce no symptoms; that 1 gram per kilo does produce symptoms and may be fatal, as also may be repeated smaller doses; and furthermore that small doses (even 0.05 cc./kilo) which produce no toxic symptoms, nevertheless produce a fatty degeneration of the liver and kidneys which is definitely demonstrable microscopically. Such findings, especially in the case of the liver, might inferentially be expected through the close relation between carbon tetrachloride and chloroform (17). The histological lesions are greatest thirty-six to forty-eight hours after administration of the drug, and repair is rapid, usually being completed after two to three weeks.

It is apparent from the above that, in the case of normal individuals, the fatal, or even toxic, dose is far in excess of the efficient therapeutic dose as an anthelmintic. On account of the undoubted toxicity of the drug, and variation in the reported results of its toxicity in animals, it is of interest to ascertain the minimum toxic dose. The methods heretofore used have been to take histological changes in the liver and kidneys—together with physical signs and symptoms—as an indication of toxicity. As the drug affects the liver more extensively than other organs, and as we have a method for testing liver function without injury to the subject, the following experiments have been undertaken using the phenoltetrachlorophthalein test as an index of functional change in the liver.

THE TEST

It has recently become possible to determine readily a change in at least one function of the liver, by noting the rate at which phenoltetrachlorophthalein disappears from the blood plasma. Abel and Rowntree (1) in 1910 found that this dye under normal conditions was excreted solely by the liver; early attempts were made to use the dye as a test for liver function, examination of stools or duodenal contents being made to determine the amount of dye present; these methods proved cumbersome and but partially satisfactory. S. M. Rosenthal (10) in 1922 devised a liver test in which the rapidity of disappearance of the dye from the blood plasma was used as an index of hepatic function; he has shown (11) that the dye is retained in the blood in cases of experimentally induced lesions of the liver, and clinically that abnormal curves are present in cases of naturally occurring pathological conditions of the liver (12).

EXPERIMENTS

In the present series of experiments, local anesthesia (1 per cent cocaine-HCl) was used in making the necessary incisions, in order to avoid the effect of ether upon liver function (16). Small buttonhole openings were made in dogs exposing the external jugular and basilic vein; after withdrawal of a 10 cc. sample of normal blood from the jugular, 5 mgm./kilo of phenoltetrachlorophthalein (prepared in 2 cc. ampoules by Hynson, Westcott and Dunning, Baltimore) made up in normal saline, were injected in the basilic vein, and 4 cc. samples of blood withdrawn from the jugular at intervals of three, ten, fifteen and thirty minutes thereafter. The dogs were then given Merck's "highest purity, 'C.P.'" carbon tetrachloride by stomach tube. The clotted bloods were centrifuged and the clear serum pipetted off; hemolysis was avoided by the use of hard glass test tubes and scrupulous cleanliness; lipemia was prevented by not feeding the dogs the evening and morning before the experiment.

The preparation of standards and the reading of the values of the unknown sera were done as outlined in Rosenthal's second paper (11); we have found that with clear sera very accurate readings can be made without difficulty.

FINDINGS

a. Liver function

1. *Dogs treated with a single dose of 4 cc./kilo.* Dog I, a male weighing 7.15 kgm.; II, female, 7.0 kgm.; III, female, 7.2 kgm. Dogs kept in cages and observed several times a day, especially for evidences of vomitus. Throughout the experiment the dogs were well, frisky, wagged their tails, and moved about readily; toward the end of the experiment they were somewhat quiet, due to confinement, but evidenced no symptoms of intoxication. The dogs were sacrificed in other experiments on the sixth and seventh days.

Curves were obtained from this series of dogs as shown in figure 1. In all three cases definite functional damage was produced forty-eight hours after administration of the drug, as shown by the persistent elevation of the curves (I.2, II.2, III.2); this action, however, was quite transient, for it had entirely cleared up within ninety-six hours after administration, the curves taken at this time (I.3, II.3, III.3) falling with the normals (I.1, II.1, III.1).

2. *Dogs treated with a single dose of 2 cc./kilo.* Dog IV, a female weighing 5.4 kgm.; V, female, 5.35 kgm.; VI, female, 6.5 kgm.; VII, female, 12 kgm; XIII, female, 9.2 kgm.; IX, female, 6 kgm.; X, male, 9.2 kgm.; XI, male, 11.2 kgm. Care of dogs as above; no symptoms of intoxication. Dog IV was quite frail and died during a very cold night, sixty hours after start of experiment; there was some bleeding, possibly from scratching of the exposed jugular. Dog V vomited the drug within half an hour, and the dose was readministered; in this case the amount of carbon tetrachloride retained from the first administration was questionable; the forty-eight hour curve, however, showed no retention of dye. The dogs were sacrificed in other experiments, subsequently.

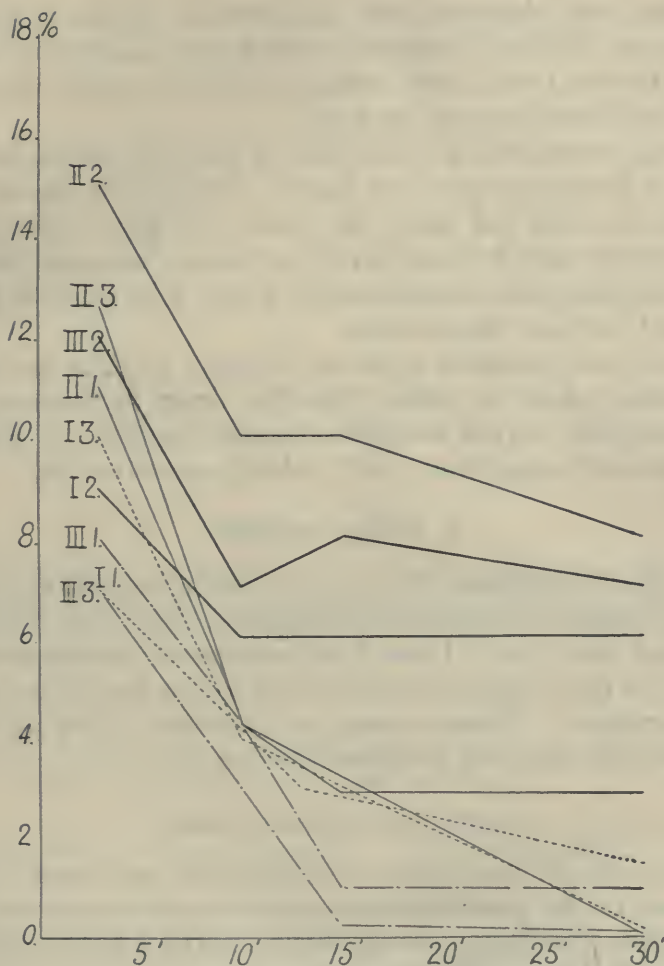


FIG. 1. SHOWING RETENTION OF DYE IN THREE DOGS GIVEN A SINGLE DOSE OF CARBON TETRACHLORIDE (4 CC. / KILO)

Curves I.1, II.1, III.1, are the normals; curves I.2, II.2, III.2, taken forty-eight hours after administration of the drug, show definite retention of the dye; while curves I.3, II.3, III.3, taken ninety-six hours after administration of the drug, demonstrate complete return to normal function.

Curves were obtained from this series as shown in figure 2. The curves obtained forty-eight hours after administration are almost identical with their normals, and demonstrate clearly no functional disturbance of the liver.

3. *Dogs treated with a divided dose of 4 cc./kilo, administered in 2 cc./kilo doses forty-eight hours apart.* Dog IX, a female weighing 6 kgm.; X, male, 9.2 kgm.; XI, male, 11.2 kgm. Dogs cared for as above; were well and lively; no toxic symptoms observed up to nine days after administration of first dose, when they were sacrificed in other experiments.

Curves were obtained as shown in figure 3. The forty-eight hour curves show no effect from the drug; furthermore, the ninety-six hour curves also show normal function. A series of tests run on the eighth day still yielded normal curves.

b. Kidney function

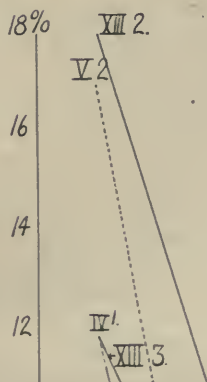
In order to ascertain effect of the carbon tetrachloride upon kidney function, phenolsulphonephthalein tests (13) also were conducted upon dogs III and V; in neither case was there retention of this dye, function being normal during the whole time of the experiment. Albumin tests on the urine of dog III were negative, and were not performed on dog V.

SUMMARY AND CONCLUSIONS

1. The toxic effects of carbon tetrachloride have been studied by means of the phenoltetrachlorophthalein liver function test. In two cases the effect on renal function has also been studied by means of the phenolsulphonephthalein test.

2. It was found that single doses of 4 cc./kilo of carbon tetrachloride produce functional disturbance of the liver in the dog, with complete return of function to normal within ninety-six hours. Signs of intoxication in these animals could be observed by this method before any visible signs or symptoms were evident. The kidneys did not appear to be effected by this dose.

3. Administration of 2 cc./kilo produced no demonstrable disturbance in either liver or kidney function.



ERRATA

Use slip below for caption to figure 2, p. 243, Vol. XXI, No. 4, May, 1923, number of JOURNAL PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS. "The Toxicity of Carbon Tetrachloride in Relation to Liver Function as Tested by Phenoltetrachlorophthalein" by Lamson and McLean.

FIG. 2. SHOWING NO RETENTION OF DYE IN EIGHT DOGS GIVEN A SINGLE DOSE OF CARBON TETRACHLORIDE (2 cc./KILO)

Curves IV.1, V.1, VI.1, VII.1, IX.1, X.1, XI.1, XIII.1, are the normals taken before the administration of the drug; curves IV.2, V.2, VI.2, VII.2, IX.2, X.2, XI.2, XIII.2, are taken forty-eight hours after administration. In the case of Dog XIII which was given a second dose of 2 cc./kilo, it will be noted that there is no retention of dye except in XIII.2, which is taken forty-eight hours after the first administration of 2 cc./kilo, but that two days later, after giving more carbon tetrachloride, the curve has returned to normal. We have no explanation of this one forty-eight hour curve.

six hours after the administration of the first 2 cc. / kilo, and forty-eight hours after the second 2 cc. / kilo. It will be noted that there is no retention of dye except in XIII.2, which is taken forty-eight hours after the first administration of 2 cc./kilo but that two days later after giving more carbon tetrachloride the curve has returned to normal. We have no explanation of this one curve.

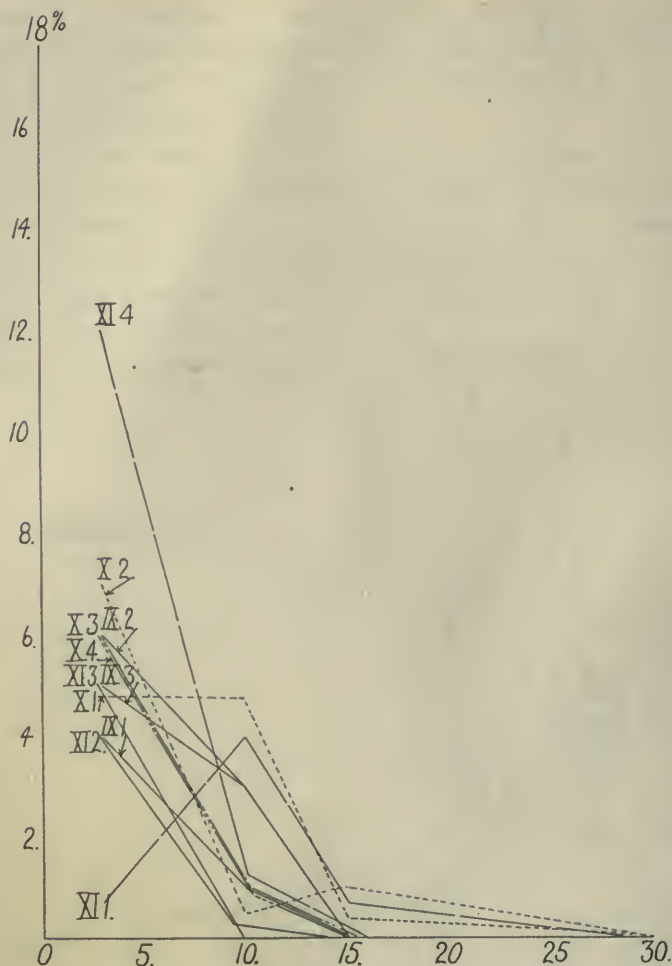


FIG. 3. SHOWING NO RETENTION OF DYE IN THREE DOGS GIVEN 4 CC./KILO OF CARBON TETRACHLORIDE, DIVIDED INTO TWO 2 CC./KILO DOSES ADMINISTERED FORTY-EIGHT HOURS APART

Curves IX.1, X.1, XI.1, are the normals taken before the administration of the drug; curves IX.2, X.2, XI.2, are taken forty-eight hours after the administration of the first 2 cc./kilo; curves IX.3, X.3, XI.3, are taken ninety-six hours after administration of the first 2 cc./kilo and forty-eight hours after the second 2 cc./kilo. Curves X.4, and XI.4, are taken nine days after the first administration of the drug. It will be noticed that there is no retention of dye in any of these cases.

4. Finally, 4 cc./kilo (the found toxic dose), given in divided doses of 2 cc./kilo at forty-eight hour intervals, were found to have no toxic effect, which is contrary to the belief that divided doses are more toxic than a single massive dose.

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THE SALICYLATES. XIV. LIBERATION OF SALICYL FROM AND EXCRETION OF ACETYLSALICYLIC ACID

P. J. HANZLIK AND ELIZABETH PRESNO

From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco

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Pharmacologically, acetylsalicylic acid differs sufficiently from sodium salicylate to indicate that the differences in action are due in part, at least, to the undecomposed acetylsalicyl group which differs chemically and physically from the ordinary salicyl group. However, it has not been conclusively demonstrated that the acetylsalicyl group can pass through the body unchanged. It is generally assumed that it is completely transformed into the salicyl group in the alimentary tract, and the effects after absorption, therefore, would be due to sodium salicylate. The extent of the decomposition would depend mainly upon the degree of chemical reaction (hydrogen ion concentration) and time of sojourn in the alimentary tract and partly upon dosage.

The chief differences that characterize acetylsalicylic acid from sodium salicylate in its actions are: (a) smaller clinical "toxic" dose for the production of symptoms of salicylism, (b) better analgesia, (c) edema-like effects, especially of the lower eyelids, and (d) other peculiar, idiosyncratic or anaphylactoid effects, which do not occur with sodium salicylate. If it could be shown that the acetylsalicyl group passes through the body unchanged, some of these differences might be attributed to its greater lipid solubility and, therefore, greater permeability and pharmacological activity.

It is the object of this paper to discuss the liberation of salicyl from, or in other words, the decomposition of, acetylsalicylic acid in vitro as influenced by the chemical reaction and time

element at body temperature, and its excretion in urin under controlled conditions. Briefly, the results that were obtained show that acetylsalicylic acid does not decompose completely within the range of chemical reaction possible in the alimentary tract, and that it is excreted unchanged to a considerable extent in the urin. First, the results on the liberation of salicyl from acetylsalicylic acid in vitro will be presented.

I. LIBERATION OF SALICYL FROM ACETYLSALICYLIC ACID IN VITRO

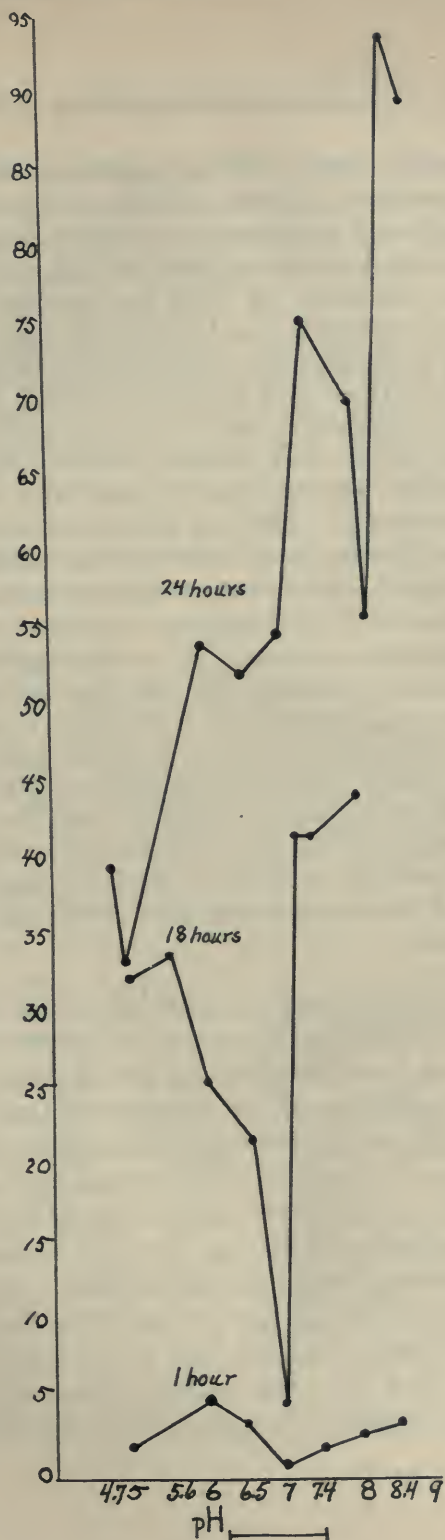
This was studied by incubating buffer mixtures of known hydrogen ion concentrations and acetylsalicylic acid at body temperature for different periods of time. The liberated salicyl was determined quantitatively by the colorimetric method of Thoburn and Hanzlik (1) without distillation and using ferric alum as the indicator. By means of the ferric ion it is possible to distinguish between the salicyl and acetylsalicyl groups. That is, acetylsalicylic acid does not give the pink to violet color of salicylic acid with the ferric ion; hence, if the test is positive, this denotes the presence of salicyl. The acetylsalicylic acid which was used in all of our experiments was found to be free from salicylic acid by means of the ferric alum test. Therefore, the positive salicyl reactions obtained in the incubation experiments were attributed to salicyl liberated as a result of the conditions in the experiments. In detail the incubation experiments were carried out as follows.

Two kinds of buffer mixtures were used. In 4 experiments the standard phosphate mixtures of Sørensen (2) were used and in 3 experiments the citric-phosphate mixtures of Mellvaine (3). The pH values ranged from 5.0 to 9.0, i.e., from acidity possible in the gastric juice to a degree of alkalinity slightly higher than that possible in the intestinal

FIG. 1. LIBERATION OF SALICYL FROM, OR DECOMPOSITION OF, ACETYLSALICYLIC ACID (0.1 PER CENT) IN PHOSPHATE AND CITRIC-PHOSPHATE "BUFFER" SOLUTIONS AT 38°C. AT THE END OF ONE HOUR, EIGHTEEN AND TWENTY-FOUR HOURS

Composite curves from values of 7 experiments (one hour) and 2 experiments (twenty-four hours); 1 experiment (eighteen hours); |—| = approximate region of minimal salicyl liberation, or decomposition of acetylsalicylic acid.

Percent Salicyl Liberated



juices. The results with the citric-phosphate mixtures were exactly the same as those with the phosphate buffers. One-tenth of a gram of dry acetylsalicylic acid was placed into each of a series of 100 cc. flasks which were then filled to the mark with the buffer mixtures. The mixtures were then incubated at 38°C. for one hour (7 experiments,) eighteen hours (1 experiment) and twenty-four hours (2 experiments). The pH values of the mixtures were obtained immediately before and after incubation. These were invariably lowered as a result of the addition of the acetylsalicylic acid. At the end of incubation, 25 cc. portions of each mixture were acidified with 10 per cent hydrochloric acid and the acidified solutions were extracted with ether in separatory funnels until salicyl-free. This was determined by evaporating 2 or 3 cc. of the last ethereal extract to dryness and adding 1 or 2 drops of water and a drop of 5 per cent ferric alum solution. The combined ethereal extracts of each mixture were evaporated and the remaining residue was made up to a volume of 50 cc. with distilled water. Salicyl was estimated colorimetrically with the ferric alum solution, using sodium salicylate as standard and containing 0.1 mgm. per 1 cc. of salicylic acid. The salicyl could not be estimated directly after incubation without ethereal extraction because ferric alum forms a precipitate with the phosphate and this interferes with the colorimetric estimation. The results of the 10 experiments that were performed are summarized in the form of curves in the accompanying figure, using median values of all experiments in cases where more than one experiment was performed.

It is seen that acetylsalicylic acid behaves rather uniquely in buffer mixtures of different hydrogen ion concentrations, namely, that salicyl from it is liberated to a greater extent in more acid (pH = 5) and alkaline (pH = 9) mixtures than in the vicinity of neutrality (pH = 7.0), including the slightly alkaline reaction of blood (pH = 7.2 to 7.4). This result is of great practical significance for our problem, since it foretold the excretion of unchanged acetylsalicylic acid in the urin of the excretion experiments to be described in the next section. At the end of one hour's incubation at 38°C., it was found that the decomposition of acetylsalicylic acid increased from 2.1 to 5.2 per cent (median values) on the acid side, i.e., between the range of pH = 4.8 to 5.8. In the vicinity of neutrality (pH = 7.0) and of slight

alkalinity ($\text{pH} = 7.5$) the liberation of salicyl was much less, namely, 1.4 per cent at $\text{pH} 7.0$ and 2.1 per cent at $\text{pH} = 7.5$. The decomposition again increased with the increase in alkalinity ($\text{pH} = 7.5$ to 8.4), but was somewhat less than on the acid side, namely, 3 per cent at $\text{pH} = 8.5$.

Qualitatively, the same general tendency in salicyl liberation at different pH levels occurred in the mixtures that were incubated for eighteen hours. This tendency was much less prominent, though still demonstrable, at the end of twenty-four hours. Quantitatively, however, the percentage decomposition was much greater at the end of eighteen hours and twenty-four hours in any given pH mixture, as would be expected. The single experiment with eighteen hours incubation showed a liberation of about 33 per cent at $\text{pH} = 5.0$; somewhat more, i.e., 34 per cent at $\text{pH} = 5.5$, and then there was a gradual diminution with the approach to neutrality, namely, a liberation of 5 per cent only. At $\text{pH} = 7.4$ (blood reaction) the liberation was about 42 per cent and this was increased to 45 per cent only at $\text{pH} = 8.0$. Two experiments were made with twenty-four hour incubation. These showed a marked increase in salicyl liberation at $\text{pH} = 4.8$ and 5.0 , namely, 40 per cent, and 33 per cent, respectively; a further increase (55 per cent) at $\text{pH} = 6.0$, then a diminution to 52.5 per cent at $\text{pH} = 6.5$ and again an increase (55 per cent) at $\text{pH} = 7.4$. At $\text{pH} = 8.5$ the liberation was 95 per cent, or, in other words, decomposition of the acetylsalicylic acid at this time was nearly complete, with some variations in solutions with pH values from 7.0 to 8.5 .

Contrary to current conceptions, these results indicate that acetylsalicylic acid is quite stable in buffer media at body temperature, requiring over twenty-four hours for complete decomposition with liberation of salicylic acid, a period much longer than the probable sojourn of the drug in the alimentary tract. It is further indicated that acetylsalicylic acid would be decomposed to a greater extent in the stomach immediately after administration than in the intestine, and that the decomposition would increase with the sojourn of the agent in the alimentary tract, leaving more than 55 per cent undecomposed at the end of

eighteen hours and about 5 per cent undecomposed at the end of twenty-four hours in the intestine, providing the reaction is at an optimum of $\text{pH} = 8.0$ to 8.5 or thereabouts. Of much greater interest, of course, is the decomposition with salicyl liberation at the end of one hour, and while absorption of the drug is proceeding.

At the end of one hour, about 95 per cent of the agent would exist in the form of the undecomposed sodium salt, i.e., sodium acetylsalicylate. During this time absorption of the salt would take place. Therefore, unchanged sodium acetylsalicylate should be present in the circulation. Since the decomposition is retarded at a pH level of 7.4 (reaction of blood), decomposition would not be favorably influenced in the blood as far as the reaction is concerned, and about 97.5 to 99 per cent of the absorbed acetylsalicylic acid (really sodium acetylsalicylate) should remain unchanged in the blood stream. Therefore, unchanged acetylsalicyl should appear in urin and this was actually found to be the case as indicated by the excretory results to be described presently. Other factors, besides reaction, namely, ferments, which may operate in the decomposition of the acetylsalicyl group, would tend to reduce the quantity in urin to a lower level than would be indicated by the quantity circulating in the blood. However, the technical difficulties of estimating small amounts of acetylsalicyl in the blood are too great to obtain reliable results. Instead of attempting this, we contented ourselves by searching for undecomposed acetylsalicyl in the urin. If undecomposed acetylsalicyl could be demonstrated in the urin, this would be reliable evidence that the agent passed through the circulation and tissues unchanged. This evidence was obtained, and, as indicated earlier in this section, was expected from the results with buffer mixtures in vitro. The excretory results may now be described.

II. EXCRETION OF UNCHANGED ACETYLSALICYLIC ACID IN URIN

Methods

The excretion was studied quantitatively in rheumatic and convalescent non-rheumatic patients selected for us by Dr.

Russel Van Arsdale Lee at the San Francisco City and County Hospital. The patients remained in bed during the progress of the experiments and received no other medication besides acetylsalicylic acid. Acetylsalicylic acid was administered in wafers containing 0.5 gram, each patient receiving 2 wafers or 1 gram of the drug every hour until symptoms of "toxicity" appeared, that is, nausea, dizziness, ringing in the ears, etc. Then the administration was stopped. At the same time urin was collected until salicyl-free. This occurred usually at the end of three to six days (median, $4\frac{3}{4}$ days) after administration was begun. The completion of excretion was determined by acidifying and extracting with ether a small portion of the last urin specimen and applying ferric alum (2 per cent solution) to the residue. If the residue failed to give a pink to violet color with ferric alum, the collection of urin was stopped. The bottles into which urin was collected contained a little chloroform as a preservative in order to prevent decomposition of the acetylsalicyl by fermentation, hydrolysis, growth of molds, etc., on standing. The urins were subjected to analysis for free salicyl, unchanged acetylsalicyl and total salicyl as soon as possible after collection.

Total salicyl: An aliquot portion of each twenty-four hour specimen was made alkaline with 10 per cent sodium hydroxide and hydrolyzed by gentle boiling under a reflux condenser for one hour. It was then acidified with 85 per cent (syrupy) phosphoric acid and distilled with steam and the distillate was estimated colorimetrically according to the method of Thoburn and Hanzlik previously described. The salicyl recovered in this way represented the total salicyl excreted, including the salicyl liberated from acetylsalicyl in its passage through the body and also any undecomposed acetylsalicyl, which was converted to ordinary salicyl during the hydrolysis.

Another aliquot portion was acidified with 10 per cent hydrochloric acid and extracted with a mixture of ordinary ether (ethyl) and ethyl acetate until a test portion of the ethereal extract (5 cc.) when hydrolyzed with 10 per cent sodium hydroxide and acidified with 10 per cent hydrochloric acid was salicyl-free as indicated by a negative ferric alum test. The ethereal extraction was carried out in the continuous percolation apparatus described by De Eds (4). In this apparatus, which is suitable for the extraction of liquids by solvents lighter

than water and gives quantitative recoveries of very small quantities of different salicyl compounds added to urin, the ethers were allowed to percolate slowly and continuously without attention through a column of urin in an ordinary drug percolator when large volumes were used and through a smaller percolator, or cylinder, when quantities of 25 to 50 cc. were used. The supernatant ethereal layer returned automatically to the extraction flask, which was heated on an ordinary electric hot plate. When the ethereal extract was found to be free from salicyl, the extraction flask was detached and the ethereal extract was allowed to evaporate spontaneously at room temperature. The residue was then rubbed down, dissolved in water and diluted to a definite volume convenient for accurate estimation of the salicyl. For small quantities of salicyl, which were known to be present in the urin by preliminary analysis with the direct distillation method, the total volume used was 25 to 50 cc.; for higher concentrations of salicyl the volume was 100 cc. An aliquot portion of the diluted extract was made alkaline with 10 per cent sodium hydroxide and hydrolyzed by boiling for one hour; then it was acidified with phosphoric acid and distilled and estimated in the usual manner. The results obtained in this way represented total salicyl and agreed with the results obtained by direct hydrolysis and distillation, and served as duplicates of the determination. Occasionally discrepancies in the results by the two methods occurred, presumably due to insufficient time allowed for complete extraction. That is, the imperfections, if any, were in the ethereal extraction method, as would be expected, rather than by the direct hydrolysis-distillation method. In any case, the disagreements were too slight to impair the value of the results for our purpose. Extraction by a mixture of ether and ethyl acetate was found to be more complete than with ether alone. This will explain discrepancies in the first 2 experiments that were performed and in which ether alone was used. With some urins, residues from the ethereal extracts were nearly colorless or very lightly colored and permitted estimation with ferric alum directly without steam distillation. However, the ethereal extracts from the majority of urins were colored and required distillation before the salicyl could be estimated.

Unchanged acetylsalicyl: Since the acetylsalicyl group does not give a positive test, i.e., pink to violet color, with ferric alum, and the salicyl group gives a positive test, it is possible to differentiate between the two by means of the iron test. The amount of salicyl excreted as acetylsalicyl was found, therefore, by difference between the salicyl of the

hydrolyzed and unhydrolyzed portions of the residue from ethereal extraction. On hydrolysis with strong alkali (10 per cent sodium hydroxide) the acetylsalicyl group is completely converted into ordinary salicyl, i.e., sodium salicylate, which on acidifying liberates free salicylic acid. In detail the steps in the analysis were carried out as follows:

An aliquot portion of the same dissolved residue from ethereal extraction, which was used for estimating the total salicyl, was slightly acidified with 10 per cent HCl so as to liberate the salicyl and acetylsalicyl as acids. If the color of the extract was not too dark so as to interfere with the colorimetric estimation with ferric alum directly, it was matched against the standard consisting of sodium salicylate in the usual way. On the other hand, if the extract was darkly colored, it was necessary to re-extract the acidified portion with a mixture of ether and ethyl acetate. The ethers were allowed to evaporate spontaneously and the residue remaining behind was made up to a convenient volume with distilled water and estimated colorimetrically in the usual way. The result obtained constituted free salicyl liberated from acetylsalicyl in its passage through the body. The difference between the total salicyl obtained after hydrolysis of the ethereal extract, or by direct distillation, and the free salicyl constituted unchanged acetylsalicyl, and in every case this was considerable as indicated by the excretory results to be described presently.

RESULTS

The excretion results that were obtained are summarized and presented together with other pertinent data in the accompanying table. Six different patients served for the study. Four of these were convalescents and two suffered with rheumatic fever. Since there were no differences between the excretory results of rheumatic and non-rheumatic individuals they will be described together. The dosage of acetylsalicylic acid administered ranged from 4 to 14.8 grams.

It is seen that the excretion of total salicyl and unchanged acetylsalicyl varied in the different individuals. For example, V. L., receiving 14.8 grams of acetylsalicylic acid, excreted a total of 68.5 per cent, while R. L. B. (a) receiving 9 grams excreted a total of 82.5 per cent, and D. V., receiving 9 grams excreted only 27 per cent. The cause of this variation was not

studied, but is thought to be due partly to expulsion of unabsorbed acetylsalicylic acid and partly to destruction of the salicyl in the same way as was found to be the case with sodium salicylate previously reported. It was not due to incomplete collection of urin since the urins were collected until salicyl-free. Quantitative recovery from the feces was not attempted. About 30 per cent of the total salicyl was found to be excreted during the first twenty-four hours after administration was begun, and from three to six days (median $4\frac{3}{4}$ days) were necessary for completion of the excretion by all the patients that were studied.

The differences between the hydrolyzed and unhydrolyzed portions of the ethereal extracts indicate that considerable salicyl was excreted as acetylsalicyl in the urin. This also varied in different individuals, and the variations bore no relation to the dosage and urinary output, which also varied. For instance, D. V., R. L. B. (a) and R. L. B. (b) received 9, 12 and 9 grams of acetylsalicylic acid, voided 6109, 9931 and 8767 cc. of urin, and excreted 36.6, 11.4 and 25.21 per cent of acetylsalicyl, respectively. The total acetylsalicyl excreted by these patients, whose urins were extracted with ether and ethyl acetate, was greater, namely, a range of from 11.4 to 36.6 per cent, than that in the urins of V. L., N. J., and S. G., which were extracted with ether alone and yielded 8.8 to 22.7 per cent. The excretion by G. R. was incomplete because of incomplete collection of urin. These results, therefore, confirm the prediction made from the results of experiments with acetylsalicylic acid in "buffers" *in vitro*. However, a knowledge of the total percentage excretion gives only a part of the desired information.

The information obtained regarding the distribution of salicyl and acetylsalicyl in the total salicyl excreted is equally interesting and significant. It gives a better idea of the fate of the absorbed acetylsalicyl group in its passage through the tissues than the total excretion. Total excretion may be modified by such factors as loss of unabsorbed acetylsalicylic acid in feces, perhaps excretion in the sweat, destruction of total salicyl in the tissues, etc. The results in the accompanying table show that a considerable proportion of any given urinary salicyl residue obtained

TABLE 1

Excretion of salicyl and acetylsalicyl in urin

PATIENT AND DIAGNOSIS	TOTAL DOSEAGE OF ACETYL-SALICYLIC ACID ADMINISTERED		TOTAL VOLUME URINE COLLECTED		TOTAL SALICYL EXCRETED (BY DISTILLATION AND HYDROLYSIS)		TOTAL SALICYL EXCRETED (BY ETHERAL EXTRACTION AND HYDROLYSIS)		TOTAL SALICYL EXCRETED AS SALICYL (BY ETHERAL EXTRACTION WITHOUT HYDROLYSIS)		PROPORTION ACETYL-SALICYL PRESENT IN TOTAL SALICYL EXCRETED		PER CENT ACETYL-SALICYLIC ACID EXCRETED OF TOTAL ADMINISTERED	TIME OF COMPLETED EXCRETION <i>hours</i>	REMARKS
	As such <i>gms.</i>	As salicylic acid <i>gms.</i>	<i>cc.</i>	Grams	Per cent*	Grams	Per cent*	Grams	Per cent	Grams	Per cent				
V. L. (acute rheumatic fever)	14.8	11.39	4,075	7.81	68.53	0526.77	2.03	66.5	1.02	33.4	8.8	120	Extraction with ether only. Vomited twice during administration of drug.		
N. J. (chronic arthritis)	7.8	6.0	8,031	4.81	80.22	1135.2	1.48	70.1	0.63	31.0	10.5	144	Extraction with ether only		
S. G. (chronic tonsillitis and myocarditis; hyperthyroidism)	4.0	3.08	5,492	3.12	101.42	7489.0	2.04	68.4	0.70	25.5	22.7	72	Extraction with ether only		
G. R. (chronic eczema)	7.0	5.39	2,054	1.71	31.71	5528.7	1.47	94.54	0.82	5.36	1.52		Incomplete collection of urine. Excretion with ether and ethyl acetate		
D. V. (cerebrospinal syphilis)	9.0	6.93	6,109	1.87	27.01	7725.65	1.53	85.07	0.25	14.30	36.6	117	Extraction with ether and ethyl acetate		
R. L. B. (a) (acute rheumatic fever)	12.0	9.24	9,931	5.49	59.54	8552.5	4.29	88.1	0.55	21.4	11.4	114	Extraction with ether and ethyl acetate		
R. L. B. (b) (acute rheumatic fever)	9.0	6.93	8,767	5.70	82.54	3462.7	2.58	59.5	1.76	41.1	25.21	96	Extraction with ether and ethyl acetate		

* Of total administered.

by ethereal extraction consisted of unchanged acetylsalicyl, ranging from 5.3 to 41.1 per cent. That is, up to four-tenths of the total absolute quantity of salicyl, represented as 100 per cent, consisted of unchanged acetylsalicyl. This proportion of acetylsalicyl did not go parallel with the total percentage excretion of acetylsalicylic acid administered. For instance, the acetylsalicyl of the total salicyl excreted by D. V. was 14.3 per cent and the total acetylsalicyl excreted was 36.6 per cent, while the acetylsalicyl portion of the total salicyl excreted by R. L. B. (a) was 21.6 per cent and the total acetylsalicyl excreted was only 11.4 per cent. The data from all patients were arranged in several ways to see if the distribution of salicyl and acetylsalicyl could be correlated with various factors, viz., clinical condition, dosage, urin output, total salicyl and total acetylsalicyl excreted, but no correlation could be found. By elimination, therefore, it would appear that the variability in distribution was due to variable influences of the tissues in different individuals on the transformation of the acetylsalicyl group into salicyl.

The salicyl obtained by difference with the ferric alum reaction and called acetylsalicyl in these experiments cannot be a conjugated form of salicyl, such as salicyluric acid, or some other form, because salicyluric acid and other forms that have been described (5) react with the ferric alum just like ordinary salicyl, giving a pink to violet color, and therefore, would be accounted for in our estimations for salicyl. In a previous study (6) salicyluric acid could not be detected by several different methods applied to the urins of animals and rheumatic and non-rheumatic individuals. Although we have made no tests for salicyluric acid in the urins of the present study, there is no reason to believe that it would occur after the administration of acetylsalicylic acid any more than after the administration of sodium salicylate. Therefore, there is no doubt, that the quantitative difference in salicyl between the hydrolyzed and unhydrolyzed portions of the ethereal extracts of the urins in the present study represents the unchanged acetylsalicyl group.

The quantity of unchanged acetylsalicyl appearing in the tissues is sufficient (in fact, very considerable) to indicate that

the differences in pharmacological action and clinical effects of sodium salicylate and acetylsalicylic acid are dependent on the different forms in which these agents are present in the circulation and tissues. That is, sodium salicylate and also completely decomposable derivatives of salicyl, appear in the circulation and tissues as sodium salicylate, whereas the administration of acetylsalicylic acid results in the appearance of both sodium salicylate and sodium acetylsalicylate.

Concerning the excretion of acetylsalicyl after the administration of small doses (0.5 to 1 gram) of acetylsalicylic acid, no data by the present methods are at hand. In some preliminary experiments (previously reported (7)) with doses of 0.5 and 1 gram, the mean excretion of total salicyl in 3 normal individuals was found to be about 60 per cent. Unfortunately, only the distillation method was employed, and, therefore, no accurate idea of undecomposed acetylsalicyl in the urin was obtained. However, there is no reason to believe that it cannot occur except for the proportionate reduction in the concentration and quantity, as compared with the rather high excretion after large doses reported in this paper. In view of the more satisfactory and convincing results obtained with larger doses (4 to 14.8 grams) in the present study, work with smaller doses has not been pursued further. Moreover, the question had been investigated in dogs by Pitini (8) and in persons by Devrient (9) while our work was in progress. Both of these authors used extraction methods somewhat similar to the method employed by us in this study, though without ethyl acetate and obvious controls. Lack of details in their papers makes it difficult to judge the precision and accuracy exercised in the manipulation of the urins and the extracts. They do not appear to have made complete extractions. For instance, Devrient recovered only 3.41 and 4.8 per cent of total salicyl from the urins of 2 persons each of whom received 2 grams of acetylsalicylic acid. This seems too small; we extracted a total of 89 per cent with a dosage of 4 grams, the smallest dose used by us. Pitini appears to have made his quantitative estimations only on the first 24 hour specimens of urins after administration of 1 to 1.5 grams of ace-

tylsalicylic acid to the 6 dogs that were used. We found that small and large doses administered to persons require three to six days for completion of the excretion. However, despite the discrepancies and uncertainties in the work of Pitini and of Devrient, the proportion of acetylsalicyl in the salicyl residues which they extracted (by our calculation from their results), agrees very well with our results. Pitini's residues indicate an acetylsalicyl proportion of about 19 to 30 per cent and Devrient's, about 66 to 74.6 per cent. Therefore, it appears that the excretion of the acetylsalicyl group occurs quite as readily after the administration of small as of large doses, and in both dogs and man.

CONCLUSIONS

1. The liberation of salicylic acid from acetylsalicylic acid in buffer solutions at body temperature (38°C.) in vitro was found to be somewhat variable though rather unique and of practical significance in accounting for the fate of acetylsalicylic acid in its passage through the body.

2. The liberation was found to be quite as great in a degree of acidity (hydrogen ion concentration) corresponding to that of gastric juice as in a degree of alkalinity corresponding to that of intestinal juices, but it was very much less in the vicinity of neutrality and of the very slight alkalinity of blood, that is, from pH = 6.6 or 6.8 to 7.2 or 7.4.

3. Accordingly, liberation of free salicylic acid from acetylsalicylic acid would be expected to occur in the stomach, contrary to current conceptions of, and claims made for, the drug in this direction.

4. The maximal liberation of salicylic acid from, or in other words, decomposition of, the acetylsalicylic acid in buffers was found to be about 4 per cent at pH = 8.4 and about 5.0 per cent at pH = 6.0 at the end of one hour; about 45 per cent at pH = 8.0 and 33 per cent at pH = 5.0 at the end of eighteen hours, and almost complete, i.e., 95 per cent, in buffers at pH = 8.0 at the end of twenty-four hours.

5. On the other hand, between 98 and 99 per cent of the acetylsalicylic acid remained unchanged, or undecomposed, at the end of one hour in buffers of pH = 6.8 to 7.0; about 95 per cent at the end of eighteen hours in a buffer with pH = 7.0, and about 45 per cent at the end of twenty-four hours in buffers with pH = 6.0 to 7.0 remained undecomposed.

6. Therefore, considerable absorption and urinary excretion of the unchanged acetylsalicyl group would be expected, and this was actually found to be the case in a quantitative study of the excretion in persons.

7. The administration of 4 to 14.8 grams (clinical "toxic" doses) of acetylsalicylic acid to 6 persons, of whom 4 were convalescents and 2 suffered with rheumatic fever, resulted in total excretions of from 8.8 to 36.6 per cent of the acetylsalicylic acid administered. The proportion of acetylsalicyl in the total salicyl extracted from the different urins was considerable, ranging from 5.3 to 41.1 per cent, with a median value of 25.5 per cent. The excretion bore no relation to clinical condition, dosage, diuresis, total salicyl excreted and other factors. The duration of excretion was three to six days (median $4\frac{3}{4}$ days).

8. This means that considerable unchanged acetylsalicyl circulates through the body, and presumably, therefore, explains the differences in pharmacological action, clinical "toxic" dosage and effects of sodium salicylate and acetylsalicylic acid.

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ON THE ENTRANCE OF CONVULSANT DYES INTO THE SUBSTANCE OF THE BRAIN AND SPINAL CORD AFTER AN INJURY TO THESE STRUCTURES

H. C. SYZ

*From the Pharmacological Laboratory and the Phipps Psychiatric Clinic, Johns
Hopkins University*

Some years ago (1910) Barbour and Abel (1) reported experiments extending and corroborating observations previously made by Abel which showed that in frogs convulsions can be produced by much smaller doses of acid fuchsin and in a shorter time if the anterior part of the fore-brain is removed. It seems not to be of importance whether this brain operation is done a few days before or shortly after the injection of the dye stuff. The assumption was made that by this operation inhibitory influences are removed which proceed from the cerebral lobes to subcortical centers. Thus the normal relation of excitation and inhibition is disturbed and it requires but little of the drug to break down the remaining barrier to the spread of reflex reactions in the spinal cord. Instead of acid fuchsin other dye stuffs as for example, phenol-sulphone-phthalein, naphthol yellow, tropaeolin, Basel I and Basel III, all sulphonated water soluble dyes, can be used as Macht (2) has shown in a paper from this laboratory (1912).

There are other conditions under which convulsions appear after small doses of the sulphonated dyes. Instead of the removal of parts of the fore-brain we may utilize physical exertion carried to the point of exhaustion (Barbour and Abel (1)) or cardiectomy (Joseph and Meltzer (3), 1911). In the cardiectomized animal the blood circulation is completely eliminated, the dye is no longer driven through the arterial system into the capillaries but is pumped by the surviving lymph hearts from the lymph sacs of the skin into a number of valveless veins which communicate with the meningeal vessels, as was conclusively

proven by Abel and Turner (4). The acceleration of the appearance of convulsions in this case can be explained by the relatively high concentration of the dye in the vessels of the brain and spinal cord and by an increase of the excitability of the brain and spinal cord due to the absence of oxygenated blood (asphyxia).

The absorbed acid fuchsin in the quantities employed in these experiments is changed into a colorless compound by the neutralizing power of the animal tissues. On the addition of hydrochloric acid a pink color appears which is in decided contrast to the normal color of the nervous substance. Abel (5) proved by this simple test that in all frogs that show convulsions after cardiectomy or injury to the brain, acid fuchsin is present in the brain and spinal cord.

Inasmuch as the action of convulsants in general is a subject of great importance in practical medicine and since we have in the experiments cited above a striking example of the effects of a slight injury to the cerebrum in the way of bringing on convulsions rapidly and after very small doses of otherwise relatively harmless dyes; I have undertaken at Professor Abel's suggestion to submit this whole subject to a renewed examination.

I. ABSORPTION OF ACID FUCHSIN IN FROGS THAT SHOW CONVULSIONS PRODUCED BY ACID FUCHSIN AND BRAIN-INJURY

A series of experiments was performed in order to test for the presence of the dye stuff in the brain and spinal cord of frogs that had convulsions after an injection of acid fuchsin coupled with an injury to the brain. It was hoped that one would be able to see the dye stuff on microscopical examination of the tissues of the central nervous system. Unfortunately this hope can not be realized even when the freshly acidified nervous substance shows a very marked pink color in consequence of the injection of a very considerable amount of the acid fuchsin. Therefore, one must rely entirely upon macroscopical inspection, upon the appearance of a pink color on moistening the crushed brain and spinal cord with hydrochloric acid.

For our experiments *rana pipiens* and *rana clamata* were used. We first followed the procedure described by Barbour and Abel (1) (1910).

Experiment 1. Seven-tenths milligram acid fuchsin (5 per cent solution) per gram of body weight was injected in the dorsal lymph sac of a frog. (It is customary in this laboratory to proceed as follows in injecting drugs into the dorsal lymph sac of frogs. A syringe with a rather long hypodermic needle is employed. The needle is thrust well into the muscles of the thigh and pushed upward until the point appears under the skin of the lower part of the dorsal lymph sac. The needle may then be pushed up farther when the contents of the syringe are injected into the lymph sac. On withdrawing the syringe it is found that there is no leakage whatever from the lymph sac since the road of escape for fluid is quite blocked by the muscles through which the entering needle passed. Only in this manner is it possible to make comparative experiments as cited here and in the previous papers from this laboratory on convulsant dyes.) The animal showed no reaction. Thirty minutes later the maxilla was transected just back of the eyes. The anterior third of the cerebral hemispheres is removed by this operation. During three minutes following this operation the frog showed somewhat less response to skin stimuli. Afterwards there was an increasing hyperexcitability and restlessness. Twenty-three minutes after the operation typical extensor cramps of tetanic character appeared in both the fore and hind legs, followed by clonic convulsions, then flexor and finally again extensor tetanus. The brain and spinal cord were taken out eighty minutes after the injection of the acid fuchsin and fifty minutes after removal of the anterior part of the cerebral lobes. This operation was done very quickly. First the os coccygis was transected with a pair of sharp scissors; the cut was then led on both sides along the spine from the os coccygis up to the upper maxilla, thus separating the spine from the other parts of the skeleton. Lastly I transected the vessels and membranes which connect the intestines, lungs and heart with the spine. Thus the blood supply to the central nervous system was not interfered with up to the moment when the brain and the cord together with their bony covering were separated entirely from the other parts of the body. The whole operation can be done in about thirty seconds.

Proceeding in this manner we exclude a disturbing influence from the side of the lymph hearts, as described by Abel and Turner (4) (1914) in cardiectomized frogs. The spine then was cleaned carefully from all muscular tissue which was always found to contain much acid fuchsin. Spine and skull were opened and on removing the brain and cord I avoided bringing it in contact with any other excised tissues of the frog.

The meninges then were carefully taken off, the brain and cord were slightly crushed and moistened with 5 per cent hydrochloric acid. A marked pink color appeared.

It has been mentioned that a very careful removal of the meninges is necessary to obtain a clear result. Proper forceps are necessary in this operation which is greatly facilitated by gently rolling the whole brain and cord over filter paper and at the same time holding down one or more of the larger blood vessels. In most of our experiments we found the color test to be positive in the meninges, also in cases where we did not find any trace of pink in the nervous substance. That a positive test is obtained with the blood that escaped from the relatively large meningeal vessels of the frog is not surprising. The vessels in the nervous substance itself seem either not to be filled with blood after brain and cord have been taken out of the body, or the amount of blood (and acid fuchsin) remaining in the nervous substance is too small to give a positive reaction.

Experiment 2. Injection of 0.6 mgm. acid fuchsin per 1 gram of body weight into the dorsal lymph sac. Twenty-three minutes later transection of the upper maxilla was performed as in experiment 1. This operation was followed after two minutes by a restless crawling around and after four more minutes by marked convulsions with extensor tetanus. The color test was positive in brain and cord three minutes after the appearance of the convulsions.

Experiment 3. Three-tenths milligram acid fuchsin per 1 gram weight were injected. Twenty-eight minutes later the anterior third of the cerebral lobes was removed by the same operation as in experiments 1 and 2. One minute later convulsions began to appear and the central nervous system which was taken out immediately after the appearance of the first convulsion presented a slight but definite pink color on addition of hydrochloric acid.

A great number of similar experiments were done. We varied the amount of the injected dye from 0.3 to 0.9 mgm. per 1 gram body weight of the animal. The transection of the upper maxilla was done anywhere from ten to forty minutes after the injection of the acid fuchsin; the convulsions appeared from one to twenty-nine minutes after the brain lesion. The brain and spinal cord

were removed in several experiments two or three minutes after the first convulsion occurred, in other experiments we waited longer (thirty minutes). The reaction on addition of hydrochloric acid was always positive; the appearance of a marked, more rarely of a very faint pink color, which was fully developed about five minutes after the application of the acid, proved the presence of acid fuchsin in the nervous substance. We can therefore say that in frogs which have convulsions following an injection of acid fuchsin and subsequent removal of the anterior third of the brain, the dye is present in the tissues of the brain and cord.

From time to time it happens that a frog does not respond with convulsions in spite of the injection of acid fuchsin combined with the brain injury. We do not consider that any importance attaches to these occasional negative results. The conditions of experiments have not always been ideal. Winter frogs and frogs which are not kept cold enough are often little responsive and do not give exact results.

Another series of experiments was done to find out how rapidly the absorption takes place after the brain has been injured.

Experiment 4. Injection of 0.7 mgm. acid fuchsin per 1 gram body weight into the dorsal lymph sac. Thirty-seven minutes later transection of the upper maxilla in the usual manner was performed. Five minutes later before convulsions had made their appearance the brain and spinal cord were removed; they showed a slight pink color on moistening with hydrochloric acid.

Experiment 5. Injection of 0.7 mgm. acid fuchsin per 1 gram body weight. Thirty-eight minutes later transection of upper maxilla. Two minutes later, before convulsions appeared, the frog was killed and the test for acid fuchsin proved to be slightly but plainly positive.

Several other experiments of the same type were done. The head operation was performed ten to thirty-eight minutes after the injection of the dye and the animals were killed before convulsions appeared (from two to fifteen minutes after the operation). There was always a perfectly definite but slight pink reaction attesting to the absorption of a certain amount of acid

fuchsin. We may therefore say that the brain injury is always responsible for a transfer of dye stuff into the central nervous system, and that this transfer precedes the appearance of the convulsions. In all these experiments one would have expected with certainty the development of convulsions, but the frogs were killed before they could appear. The actual time which elapses between absorption and the expected convulsions can not be determined with any degree of certainty but it was found in our experiments that the dye is present in the brain and cord very shortly after the brain injury (two to five minutes) whereas the convulsions appear in some animals immediately (one minute), in others only after a longer interval (twenty-nine minutes) following the injection.

II. EXPERIMENTS WITHOUT BRAIN INJURY

Experiment 6. Three-tenths milligram acid fuchsin per 1 gram of body weight were injected into the dorsal lymph sac of a frog. The animal did not show any reaction. After thirty minutes the brain and spinal cord were taken out. There was no trace of pink color on moistening them with hydrochloric acid.

Experiment 7. Experiment 6 was repeated but with a larger dose of acid fuchsin, 0.7 mgm. per 1 gram body weight. No convulsions occurred. Brain and spinal cord showed no pink color on the usual test.

Experiment 8. The time between injection of the acid fuchsin and the removal of the brain and spinal cord was prolonged to forty-five minutes. The results were the same as in experiments 1 and 2.

Experiments of this kind were performed many times; they gave usually the results as reported above. A few exceptions will be discussed later. We thus find that the tissues of the intact brain and spinal cord do not usually absorb the acid fuchsin which is injected into the dorsal lymph sac and which from there is pumped into the circulating blood by the action of the lymph hearts.

We find a striking difference in the absorption of the acid fuchsin by the intact and by the injured central nervous system. A few minutes after the removal of the anterior third of the brain

we already see a definite absorption of the previously injected dye throughout the brain and cord, whereas the intact central nervous system seems not to be able to absorb the dye although it is circulating in the same concentration in the vascular system. The illustration will make clear this fact. In frogs A and B 0.7 mgm. acid fuchsin per 1 gram body weight were injected into the dorsal lymph sac. Frog A did not show any reaction, he was killed fifty minutes after the injection of the dye. Not a trace of pink color appeared in the nervous substance on the addition of hydrochloric acid. In frog B the brain was injured in the usual manner twenty minutes after injection of the dye. Twelve minutes after the operation, typical convulsions appeared. The marked pink color obtained with hydrochloric acid shows a definite absorption of the acid fuchsin into the central nervous system.

In frogs C and D we used phenol-sulphone-phthalein which has a convulsant effect as Macht (2) working in Abel's laboratory has shown. In frog C 0.4 mgm. of the dye stuff was injected into the dorsal lymph sac. No convulsion appeared. The brain and spinal cord were removed fifty-five minutes after the injection. They did not show any color which could have been attributed to the phenol-sulphone-phthalein. In frog D the anterior third of the forebrain was removed five minutes after injection of 0.4 mgm. of the dye per 1 gram body weight. Twenty minutes after the brain injury marked clonic and tetanic convulsions appeared and the removed central nervous system was markedly pink in color.

III. EXPERIMENTS THE RESULTS OF WHICH DO NOT COME UNDER THE ABOVE OUTLINED RULES

A few frogs on the brain of which no injury had been inflicted and in which no convulsions occurred, gave a positive color test in the nervous tissues after injection of acid fuchsin.

Experiment 9. Injection of 0.7 mgm. acid fuchsin per 1 gram body weight into the dorsal lymph sac; no convulsions. Cord and brain were removed after thirty minutes and a very slight pink color was produced by the usual test.

Experiments 10 to 12. We had the same result with a few other frogs in which the interval between injection of acid fuchsin and removal of brain and cord was prolonged to fifty and sixty-seven minutes and to twenty hours. On addition of hydrochloric acid a very faint pink color appeared.

Experiments 9 to 12 show that in exceptional cases some acid fuchsin may be absorbed by the intact central nervous system (and that without the influence of cardiectomy or of fatigue). The amount of the absorbed dye is in these animals always very small (as shown by the faint pink reaction).

In a few other frogs we got as an exceptional result the appearance of convulsions without preceding brain injury.

Experiment 13. Injection of 0.7 mgm. acid fuchsin per 1 gram body weight. Thirty minutes after the injection definite convulsions appeared although these were less marked than is usual after injury to the brain. Four minutes after the appearance of the convulsions the brain and the cord were removed; the hydrochloric acid test gave a slightly positive reaction.

Experiment 14. Two minutes after injection of 0.3 mgm. acid fuchsin per 1 gram body weight marked convulsions with typical extensor tetanus appeared. The nervous tissue showed a slight pink color four minutes later.

We know from Abel's (1) experiments that convulsions may appear spontaneously after the injection of large doses of acid fuchsin (a few milligrams per 1 gram body weight). As a rule, after doses of 1 to 4 mgm. per 1 gram of body weight the tetanus will not appear until from one to twenty-four hours have elapsed, though in three instances out of forty-four, the convulsions appeared in a half hour or less. Experiments 13 and 14 show that in rare cases convulsions without a preceding brain lesion may appear in a short time (two to thirty minutes) after the injection of the dye. The amount of the injected dye stuff was the same as in the other experiments, an amount which as a rule, is insufficient to produce convulsions in intact frogs. The amount of the absorbed acid fuchsin was not large in these instances, the coloration being about the same as in experiments 9 and 12.

The experiments reported so far show that the central nervous system of frogs absorbs regularly and very quickly the acid fuchsin circulating in the blood after ablation of the anterior third of the cerebral lobes. This absorption of the dye is followed by convulsions. It seems quite certain that the brain operation is the cause of the absorption of the dye which does not, as a rule, enter into the intact nervous substance.

There have been a few exceptional cases in which a small amount of acid fuchsin was found in the not injured nervous substance. One might object that these latter results are produced by a faulty technique. For example, a small piece of the meninges with its blood vessels might remain attached to the nervous tissue and be the cause of a positive reaction. Aware of this possibility we always carefully took off the meninges in our experiments and we think that the appearance of a pink color really means the presence of the dye stuff in the nervous tissue.

Furthermore, we observed a few animals which showed convulsions shortly after injection of the acid fuchsin (without any other manipulations) thus proving (and also by a positive pink reaction) that some acid fuchsin may be absorbed by the intact brain. The conditions which lead to this exceptional result are unknown. The occurrence of convulsions in the intact frog after small doses of the dye stuff is very rare. A slight absorption without convulsions seems to occur about twice as often as with convulsions. Although these results are exceptional we consider it important to mention them. They show that the appearance of convulsions does not depend alone upon the presence of the dye in the nervous tissue. A certain amount of acid fuchsin might be absorbed without producing the convulsive reaction. We therefore do not think that the increase of absorption is the only cause for the appearance of convulsions. We found that the pink color in several animals with convulsions was very faint, not more marked than in the few frogs which showed a positive reaction in the intact brain without convulsions. We therefore follow Abel in the assumption that the brain injury itself is a disposing factor for the occurrence of convulsions. Only

this assumption can explain the regular appearance of convulsions after brain injury even if the dye stuff is absorbed in such small amounts as do not give any convulsions in animals with an intact brain. It seems to be uncertain whether this injury to the brain produces a removal of inhibitory influences of the cortex on subcortical centres and thus facilitates the appearance of convulsions. We shall return to this point later and here only present our conclusions:

The brain injury (ablation of the anterior third of the cerebral lobes) (*a*) facilitates the absorption of the acid fuchsin by the central nervous system, and (*b*) it affects the central nervous system so that convulsions appear much more rapidly than in animals with an intact brain and cord.

In this connection reference may be made to the experiments made by Sauerbruch (6) on monkeys and rabbits in 1913. This investigator found that these animals were much more susceptible to cocaine after the motor cortex of the left hemisphere was injured. Convulsions then appeared after the subcutaneous injection of one-fifth of the amount ordinarily required to produce convulsions in uninjured animals. The same author also found—as did Barbour and Abel (1) already in 1910 in their experiments with acid fuchsin in frogs—that fatigue causes convulsions to appear after much smaller doses of cocaine. Sauerbruch suggested that the subcortical centres in the fatigued animal might respond more readily to stimuli that arise in the cortex. He did not, however, present any facts which throw light on the nature of this increased predisposition to convulsions and more especially he did not discuss the possibility of an increased absorption of the convulsant drug by the nervous tissues of the injured animal.

After the causal relation of brain injury and absorption of dye in the central nervous system has been established, the question arises how the mechanism of this relationship is to be formulated.

IV. INFLUENCE OF BRAIN LESION ON THE HEART AND LYMPH HEARTS

The acid fuchsin injected into the lymph sacs is pumped by the lymph hearts into the venous system, from where it proceeds to the heart and to the arteries. The anterior lymph hearts empty into the vena vertebralis (this vessel is not located in the intra spinal space as the name suggests) which is centrally connected with the vena jugularis- vena anonyma- vena cava anterior. The posterior lymph hearts throw the blood into the cava iliaca transversa from where the blood flows, into the vena ilaca communisrenal vessel system-vena cava inferior. Taking this way the acid fuchsin finally reaches the arteries of the meninges and the brain. Normally there is no other route from lymph sac to the spinal cord and brain. Abel and Turner (4) showed that after elimination of the blood circulation by cardiectomy the acid fuchsin takes another way. It is driven by the action of the surviving lymph hearts in a peripheral direction through the veins, i.e., in an opposite direction from that usually followed. Via anastomoses it finally passes into and distends the veins of the meninges and of the brain.

The question has to be answered whether a brain injury might influence the distribution of the dye in a similar way.

Experiment 15. The heart of a frog was exposed and the heart beats were counted. The beats were regular and the rate was 80 in a minute. Then the upper maxilla was cut through just in front of the eyes, the heart was observed; the rate was 76 to 78 in a minute. After five minutes the upper maxilla was transected back of the eyes, at the level where transection produces a removal of the anterior third of the cerebral lobes. Immediately following this operation a depression of the heart action was observed, we counted after the first minute 50 beats but two minutes later again 80 beats in a minute. During the following four minutes the action was of somewhat changing rate, varying from 74 to 94 in a minute. Afterwards the rate was found to be the same as before the operation, namely 80. The volume of the heart contractions was not changed.

According to this experiment the brain lesion causes a slowing of the heart action for a very short time (one to two minutes) with subsequent irregularity of the rate during a few minutes. This slight temporary disturbance of the heart action can not be considered as seriously interfering with the normal circulation of the blood and it can not have the same effect as cardiectomy.

Experiment 16. The beats of the posterior lymph hearts, the action of which is visible through the skin (on both sides of the posterior end of the os coccygis) were counted, the rate was found to be changeable, 120 to 150 in a minute. The anterior lymph-hearts then were exposed (they are located under the scapulae), the rate of beats was counted, 120 to 150 in a minute. Then the operation was performed at the usual place and the rate of the lymph hearts was counted again. There was some depression of their action, the rate was 80 to 100 during the following five minutes.

Experiment 17. Injection of 0.3 mgm. acid fuchsin per 1 gram body weight. One hour after this injection all four lymph hearts were exposed and burnt out; thus no more dye could enter from the lymph sacs into the circulating blood except by the slower process of diffusion. Twenty minutes after this operation the anterior part of the brain was removed and convulsions appeared immediately. The color test was positive.

Experiment 16 shows that the brain lesion has some slightly depressing influence on the action of the lymph hearts. This might cause diminished transportation of dye stuff into the blood. If the change of the action of the lymph hearts had anything to do with an increased absorption of acid fuchsin into the central nervous system we could expect such an increase of absorption only from a rise of activity with subsequent higher concentration of the dye in the blood. Our findings tend in the opposite direction and can therefore not be used for an explanation of the absorption. Moreover, it is shown in experiment 17 that the absorption of the dye by the brain and the cord takes place even if the activity of the lymph hearts is entirely eliminated, the heart itself being intact. But in this case the acid fuchsin already present in the blood is sufficient to produce convulsions. Under these circumstances the rapid absorption of

the dye by the tissues of the brain and cord is not dependent on any change in the rate of pulsation of the lymph hearts, or even on the presence of these organs. The only necessary condition for this rapid absorption appears to be this; there must be a sufficient amount of the dye inside the blood vessels of the brain and the cord at the time of the brain injury.

The above experiments then induce me to think that the slight influence of the brain operation *per se* on the action of the heart and the lymph hearts does not give us any explanation for the increase of absorption after brain injury.

V. POSSIBILITY OF ABSORPTION FROM THE INJURED REGION

In most of our experiments the region of the brain lesion was covered with blood and thus the injured nervous substance came into direct contact with the acid fuchsin present in the shed blood. We therefore have to take into consideration the following possibility of absorption. The acid fuchsin might enter into the nervous tissue at the place of injury and from there spread out by a process of diffusion throughout the entire brain and cord. Inasmuch as diffusion through such distances would require a very long time such a process seems to be very little probable. We have in the central nervous system not a system of real lymphatics. Fluid normally passes from the blood capillaries into the perineuronal and perivascular spaces and from there into the subarachnoidal space (Weed (7)). There is no indication which would lead to the assumption that there is an exchange of fluids in the central nervous system between points which are not close together. Such a process if existing, not having a mechanical propulsive force back of it, would surely not be a rapid one and it is not probable at all that by such a mechanism the dye would penetrate the entire substance of the brain and spinal cord in two minutes (as shown in our experiments). Other facts agree also against the assumption of a process of imbibition; so for example in experiments done with higher animals (Bruno (8)) it has been shown that dye stuffs applied to an injured region of the brain do not diffuse at all, or only to a slight extent, into the surrounding nervous substance.

VI. DOES THE PRESENCE OF ACID FUCHSIN IN THE SPINAL FLUID EXPLAIN THE ABSORPTION OF DRUGS?

We produce by our brain injury a marked change in the relations between blood, spinal fluid and nervous substance and one must try to look for a connection between such changes and the increased absorption of the acid fuchsin. The brain lesion causes a bleeding and subsequently a mixing of blood and cerebrospinal fluid. Thus the acid fushcin which is in the blood gets in touch with the surface of the brain and the spinal cord and there is the possibility that the dye might be absorbed from the surface of the nervous substance. There are observations in higher animals and human beings which show that certain substances have no, or only little effect, when circulating in the vessels, but that they produce a very marked reaction when injected into the subarachnoidal space or into the ventricles. (Bruno (8), Goldmann (9), Stern (10), Strecker (11).)

Experiment 18. Fifteen minutes after injection of 0.3 mgm. acid fuchsin per 1 gram body weight, we began to open carefully the skull over the two hemispheres. The animal did not show any convulsive reaction after this injury. Eighteen minutes after beginning this operation a bleeding into the intracranial space occurred. The blood came out of an open vessel of the skull, and it covered the exposed parts of the brain. The animal did not show any reaction. The meninges then were lifted up with a pincette and a small cut was made with a pair of fine scissors. Thus the blood was allowed to get into direct contact with the brain. The animal showed some restlessness but no convulsions. Five minutes later the brain and spinal cord were removed, no pink color appeared on addition of hydrochloric acid.

Experiment 19. Twenty-six minutes after injection of 0.3 mgm. of acid fuchsin per 1 gram body weight, the skull of the frog was opened over the hemispheres. We proceeded very carefully in order to avoid any bleeding. Not one drop of blood was allowed to enter into the opened skull or to get into contact with the central nervous system. Then a slight prick was given to the right hemisphere, no blood appeared on the place pricked. Five minutes after this slight injury general extensor convulsions appeared. The brain and spinal cord which were removed ten minutes later showed a diffuse absorption of acid fuchsin (a slight but definite pink coloration).

In experiment 18 some blood and dye stuff came into contact with the surface of the brain. The meninges were opened but the central nervous system was not injured. No convulsions occurred and the removed brain-cord did not show any absorption of the dye. The spinal fluid containing blood and acid fuchsin seems not to be absorbed by the nervous substance, or at least not in a very short time.

It should be mentioned that the operation as performed in experiment 18 is a rather difficult one. It often happens that the meninges are pulled, or that the brain is injured a little. On several occasions we were not quite sure whether such an accident had happened. Convulsions occurred and we think that they were due to such a slight brain lesion.

In experiment 19 no blood was allowed to get into contact with the surface of brain and spinal cord. Convulsions appeared nevertheless after brain injury. They cannot be explained by an absorption of the acid fuchsin from the spinal fluid because it is not present there. (The color test in the spinal fluid was negative.) We want to mention another important point in this experiment namely that the convulsions were produced not by the usual ablation of the anterior part of the forebrain, but by a simple pricking of one of the hemispheres.

We therefore draw the conclusion that the presence of a certain amount of dye in the spinal fluid does not explain the appearance of convulsions. An absorption of the highly diluted dye from the spinal fluid does not take place, or at least not in such a degree as we see usually in our experiments, with brain injury.

As Weed (7) has shown, a fluid stream normally passes from the perineuronal and perivascular spaces of the brain to the subarachnoidal space, a current in the opposite direction which would explain absorption from spinal fluid occurs only in exceptional cases (injection of hypertonic salt solution into the blood).

In the above reported experiments (in which the acid fuchsin was not allowed to enter into the cerebrospinal fluid) and probably in many of the others (where the dye was present in the cerebrospinal fluid also) we have to assume a direct transfer of acid fuchsin from the blood vessels into the nervous tissue.

We do not know for certain whether we have to deal with an increased permeability of the walls of the brain vessels due to the brain injury, or whether a change in the nervous substance takes place which would cause an increase of absorption.

Very little is known about the innervation of the brain vessels in the frog; it is doubtful whether there are special vasomotor nerves to these blood vessels.

According to recent publications it seems to be quite certain that changes in their innervation can cause a change in the permeability of the blood vessels although the results of the different investigators are not quite in agreement. Hoffmann and Magnus-Alsleben (12) severed the sympathetic nerve fibres to certain muscles and found, following this operation, an increase in the absorption of dye stuffs into the muscles. This increase of absorption was considered to be due to an increased permeability of the blood vessels resulting from the loss of the sympathetic innervation (dilatation?). A similar effect of the loss of their sympathetic innervation was described by Wessely (13)¹ (1908) in the vessels of the eye. He observed that fluorescein passed more rapidly from the blood vessels into the anterior chambers of the eye after the sympathetic nerve fibres had been cut through. Kajikawa (14) working in Asher's laboratory found recently that extirpation of the superior cervical ganglion caused fluorescein to enter the anterior chamber of the eye later than it did the eye of the intact side. He therefore draws the conclusion that the sympathetic innervation has an influence on the permeability of the blood vessels. The decrease in permeability after removal of the sympathetic innervation does not fit in with the above mentioned results of Hoffmann and Magnus-Alsleben (12) and of Wessely (13) who found an increased permeability after the loss of the sympathetic innervation. For us it is immaterial at the moment whether it is a nervous stimulation or depression which causes an increase of permeability, we merely state the fact that a change in the innervation of the blood vessels according to the authors cited above, causes a change

¹ Cited from Hoffmann and Magnus-Alsleben (12).

of their permeability and we see that an analogous process may possibly be involved in our experiments with frogs: that we produce by an injury to the brain a change in the innervation of the brain vessels and in that way also a change of their permeability. In this connection mention might be made of recent investigations by Krogh and Harrop (15). These investigators found that in frogs the permeability of the capillaries of the tongue is increased after their rapid dilatation. Colloidal dye (vital red) and soluble starch which are retained in normal blood vessels penetrate easily through small openings between the endothelial cells of the dilated capillaries. The dilatation in Krogh's experiments was produced by the application of a drop of 25 per cent urethane solution on the tongue. Although we do not know anything definite about a dilatation of the capillaries of the brain in our experiments, it is important to remember that such a dilatation connected with increased permeability in other parts of the vascular system has been produced experimentally by others.

As mentioned above, the injury to the brain may possibly produce an alteration in the nervous substance itself, an alteration which might consist in a widening of the perivascular or perineuronal spaces. Or we might have—as a consequence of opening the subarachnoidal spaces—a change in the pressure relations between the blood in the capillaries and the tissue fluid of the surrounding nervous substance. It was attempted to put this hypothesis to the test of experimentation but I found it exceedingly difficult to open the meninges without any injury to the underlying brain substance so that my experiments in regard to this particular point must remain inconclusive for the present.

While the various possibilities are being pointed out, we wish it to be clearly understood that we are not committing ourselves to any hypothesis for the time being.

VII. PROTECTIVE INFLUENCE OF THE MENINGES

In Experiment 18 we produced a slight injury to the meninges in order to bring blood and acid fuchsin into direct contact with the surface of the brain. It is not enough to put the dissolved

dye stuff on the intact meninges of the brain; the results are modified in this case by the highly protective function of the meninges. The following experiments may serve to show how much the meninges protect the brain against substances which are put on their outer surface.

Experiment 20. The optic lobes and the medulla oblongata of a frog were laid bare. One drop of a 10 per cent sodium chloride solution was put on the exposed but intact meninges. The frog showed some restlessness. No convulsions appeared. The meninges were opened and very marked convulsions appeared immediately without my having put any more salt solution on the exposed brain.

Experiment 21. One drop of 10 per cent sodium chloride solution was placed on the exposed but intact meninges. The frog made some restless movements at first, then he behaved quite normally. During the space of twenty minutes a few more drops were put on the meninges; the frog did not show any reaction. The meninges then were opened carefully and very marked extensor convulsions of tetanic character appeared immediately; the frog thereupon showed marked opisthotonus.

Experiment 22. The skull of a frog was opened over the optic lobes and the medulla oblongata without injuring the meninges. A drop of saturated solution of picrotoxin was placed on the meninges over the fossa rhomboidea. There was no reaction. A second drop was put on three minutes later without effect. Then the meninges were lifted and cut through; one minute after this operation very marked extensor cramps with opisthotonus appeared.

Experiment 23. The brain of a frog was exposed in the region of the lobi optici and the fossa rhomboidea. The meninges were not injured. One drop of 0.01 per cent strychnine nitrate solution was put on the meninges. The frog showed some restlessness but no convulsions. A second drop of the same strychnine solution put on two minutes later caused a slight increase in reflex excitability. After four more minutes the meninges became somewhat edematous. Then the meninges were opened and another drop of strychnine solution brought directly upon the exposed brain. Two minutes after this operation marked extensor convulsions appeared, which persisted after transection of the spinal cord below the calamus scriptorius.

Experiment 24. The skull of a frog was opened without injury to the meninges. A few drops of 1 per cent acid fuchsin were put on the

exposed meninges; the animal showed no reaction during the following fifteen minutes. Then the meninges were opened over the lobi optici. Two minutes later typical convulsions appeared.

These experiments show that the meninges in frogs are not rapidly permeable to fluids which are brought into contact with their outer surface. Sodium chloride solution brought into contact with the intact meninges causes only a certain unrest. But marked convulsions appear immediately after incision of the meninges, i.e., as soon as the salt solution comes into direct contact with the tissues of the brain. The convulsions thus produced are explained by Heubel (16) (1874) as an effect of the sodium chloride solution on locomotor centers in the region of the lower part of the fossa rhomboidea (calamus scriptorius). A similar impermeability of the meninges exists for picrotoxin, for strychnine nitrate and for acid fuchsin. We cannot say whether the meninges of the frog are impermeable for every sort of solution. At any rate, it is important to realize that such a barrier exists for certain crystalloid as well as for certain colloid solutions. It may be recalled here that (according to Ecker and Wiedersheim (17)) the dura mater in amphibia consists of a parietal (periostal) and a visceral membrane. We injured in our experiments the visceral membrane which covers the leptomeninx. The latter in frogs does not show a definite differentiation into arachnoidea and pia.

A 1 per cent solution of acid fuchsin when brought directly into contact with the surface of the brain is apt to produce convulsions, as is shown in experiment 24. This does not seem to fit in with our previous findings, namely, that the acid fuchsin which is contained in the blood that mixes with the spinal fluid in case of bleeding is not absorbed by the nervous substance. To understand these divergent results we should keep in mind the fact that in those earlier experiments the dye was highly diluted. When using a higher concentration (a 1 to 5 per cent solution) we can not expect the same negative results, as we know that the absorption by the brain and spinal cord, of minimal quantities of the dye produces convulsions. It is possible that

in the experiments with direct application of the dye to the surface of the brain the convulsions are due largely to a stimulation of superficially situated parts of the medulla (Heubel's Krampfzentrum). In this case the absorption of the dye throughout the entire nervous substance would not be necessary for the occurrence of convulsions.

VIII. INJURY TO THE BRAIN AT VARIOUS LEVELS

Experiment 25. Three-tenths milligram acid fuchsin per 1 gram body weight was injected into the dorsal lymph sac of a frog. Forty-five minutes later the upper jaw was cut through directly in front of the eyes, thus transecting the anterior part of the olfactory lobes. The frog shows some slight excitement but no convulsions. The brain and spinal cord did not show any pink staining when removed forty-five minutes after the operation.

Experiment 26. The same operation was performed ten minutes after injection of 0.7 mgm. acid fuchsin per 1 gram body weight. No convulsions appeared. The color reaction in the nervous substance was very slightly positive (thirty-five minutes after the operation).

Experiment 27. The experiment was performed in the same way as above (experiments 25 and 26). No convulsions appeared but there was a definitely positive pink reaction nineteen minutes after the injury to the olfactory lobes.

Experiment 28. Ten minutes after injection of 0.3 mgm. acid fuchsin per 1 gram body weight, a large opening was made in the skull directly over the cerebral and the olfactory lobes. No convulsions appeared. Twenty-five minutes later the left olfactory lobe was destroyed, the frog showed no reaction. Removal of the right olfactory lobe ten minutes later had no effect. Then the brain was injured in the region of the fossa limbica and ten minutes later marked convulsions appeared.

Experiments 29. Ten minutes after injection of 0.7 mgm. acid fuchsin per 1 gram body weight the skull of the frog was opened and the right forebrain pricked. Ten minutes later typical convulsions appeared and the removed nervous substance gave a pink reaction.

Experiment 30. The skull of a frog was opened and 0.3 mgm. acid fuchsin per 1 gram body weight was injected into the ventral lymph sac. No convulsions appeared. One hour later the opening in the skull was enlarged and the region between the forebrain and the

optic lobes was pricked. After two minutes marked convulsions appeared.

Experiment 31. Injection of 0.7 mgm. acid fuchsin per 1 gram body weight. One hour later the skull was opened and the brain pricked in the region of the optic lobes. Marked convulsions appeared.

In a few other experiments we obtained similar results. The level at which the transection is always effective in initiating convulsions can not be made out quite accurately. In some animals convulsions appeared if the injury was done just anterior to the fovea limbica. In other frogs we had to go a short distance posterior to this region. Injury to the olfactory lobes does not suffice to produce convulsions, the injury has to be done in the region of the anterior part of the hemispheres or further back.

IX. INFLUENCE OF INJURY TO THE MEDULLA OBLONGATA

Experiment 32. The skull of a frog was opened over the optic lobes and the fossa rhomboidea fifty minutes after injection of 0.3 mgm. acid fuchsin per 1 gram body weight. Twenty minutes later the middle portion of the medulla oblongata was punctured with a small stylet. The frog showed some depression and decreased excitability. But this lasted only a very short time (two minutes). Then the frog began to jump about; extensor tetanus with opisthotonus and clonic convulsions appeared.

Experiment 33. In a frog which showed marked convulsions after the injection of acid fuchsin into the dorsal lymph sac combined with brain injury, the skull was opened. Both cerebral lobes, the optic lobes, the cerebellum and the anterior two-thirds of the medulla oblongata were removed; the convulsions persisted. After the removal of the lower part of the medulla (section below calamus scriptorius) the spontaneous convulsions disappeared but extensor tetanus followed stimulation of the skin.

Experiment 34. Thirty minutes after injection of acid fuchsin the spinal cord was severed below the calamus scriptorius. Five minutes later the frog began to show some reflex hyperexcitability and after five minutes more extensor convulsions appeared but only after external stimuli were applied.

Experiment 32 shows that a slight injury to the medulla oblongata is effective also. Removal of the entire medulla has a

depressive effect; the convulsions if already present disappear or they only occur on stimulation from the outside. On this occasion we want to mention a few other facts. It is well known that the brain and the medulla oblongata are not essential to the convulsions induced by strychnine and that the convulsions therefore are present after removal of the brain and the medulla oblongata. Heubel (13) showed that a 10 per cent sodium chloride solution brought directly on to the surface of the medulla oblongata induces immediate convulsions, which persist after the cerebral lobes, the optic lobes and the cerebellum have been removed, but which cease immediately after the removal of the medulla oblongata. These convulsions seem to be produced by local stimulation of centers in the medulla oblongata. The acid fuchsin seems to take its place between strychnine and the sodium bromide. According to our experiments a local application of the dye stuff to the region of the fossa rhomboidea often produces immediate convulsions and one is inclined to think here of a stimulation of centers in the medulla oblongata. This assumption of special centers in the medulla oblongata is supported by the fact that after removal of the entire medulla oblongata the convulsions are considerably diminished. The convulsive moments which persist are of the type which we find after the application of strychnine, i.e., they occur especially after stimulation, whereas the convulsions with an intact medulla oblongata are not so dependent on outside stimuli. The conditions here are rather involved and we can not say that the acid fuchsin convulsions originate in strictly localized structures of the medulla oblongata but we think that these structures are in closer relation to the convulsive reactions than others.

X. TRANSECTION OF THE SPINAL CORD

Experiment 35. Four-tenths milligram acid fuchsin per 1 gram body weight was injected into the dorsal lymph sac of a frog. Ten minutes later the spinal cord was transected at the level of the seventh vertebra. No convulsions occurred during the following thirty minutes. There was a very slightly positive pink reaction (hydrochloric acid test) in the nervous substance of the cord and brain.

Experiment 36. Thirty minutes after injection of 0.7 mgm. acid fuchsin per 1 gram body weight, the spinal cord was severed at the level of the fifth vertebra. No convulsions appeared during the following forty minutes. The brain and cord showed no absorption of the dye.

Similar results to this last were obtained in several experiments with transection of the spinal cord at different levels. In a few experiments, however, we obtained convulsions as is shown in the following protocols.

Experiment 37. Three-tenths milligram acid fuchsin per 1 gram body weight was injected into the ventral lymph sac and fifteen minutes later the spinal cord was transected at the level of the fifth vertebra. One minute later the frog showed marked opisthotonus both spontaneously and after stimulation of the skin. Marked tetanic convulsions of the legs appeared. The reaction with hydrochloric acid was not done.

In a few other animals we obtained after injection of acid fuchsin combined with injury to the cord, cramp-like movements but not typical convulsions.

As above reported we transected in our experiments the spinal cord at various levels. This operation followed the injection of the dye into the dorsal lymph sac at an interval of ten to fifteen minutes. We often did not see any convulsions. The test with hydrochloric acid showed no, or only very faint, absorption of the dye.

A few experiments gave divergent results, namely, extensor convulsions and opisthotonus especially on skin stimulation. The convulsions were not as strong by far as in the experiments with brain injury. The influence of the shock of transecting the cord is probably very essential and it makes a clear understanding of the results after injury to the cord difficult.

Summarizing the results of experiments with injury to different regions of the central nervous system (after injection of acid fuchsin) we can say that injury to or removal of the olfactory lobes is not sufficient to produce convulsions. This fact was emphasized by Barbour and Abel (1) in 1910. The level where injury has to be done is at the anterior end of the cerebral lobes about in the region of the fossa limbica. Removal of the more posterior parts of the brain have the same effect as the above

noted injury. If the medulla oblongata is taken away a depressive influence on the convulsions is noticed, especially if the posterior end (region of the calamus scriptorius) is removed. This fact and the strong convulsive reaction to direct application of a more concentrated acid fuchsin to the fossa rhomboidea support the assumption that the nervous tissue in the posterior part of the medulla oblongata has something to do with regulating the motor impulses. Stimulation of this region seems to be one of the conditions necessary for the occurrence of convulsions. We mention in this connection the experiments reported by Heubel (13) which we have repeated. Direct mechanical stimulation of the posterior parts of the medulla oblongata in frogs produces cramp-like movements, fibrillary twitching, maintenance of peculiar postures.

Transection of the cord has not the same effect as brain injury. This operation seems not to facilitate absorption of the dye stuff by the brain and cord to the same degree as do the various lesions at higher levels as above described, though we got weak convulsions in a few animals. The injury to the spinal cord then seems not to have an effect quite analogous to the brain injury but our results in regard to this point are not definite enough for us to base upon them a positive conclusion.

Experiments 19, 28, 29, 30, 31 and 32 show that it is not necessary to remove a part of the brain to get a convulsive reaction. Pricking the cerebral lobes, the optic lobes or the fossa rhomboidea may be sufficient injury. Keeping in mind this fact we have to revise the conception which was presented by Barbour and Abel (1), namely, that inhibitory influences that pass from cortex to subcortical centers are removed by the brain operation. The broader statement of these authors, that the slight operation on the brain so disturbs the normal ratio of excitation to inhibition that the former now predominates, while not constituting an explanation, nevertheless, tersely describes the situation after each of the above injuries.

We content ourselves with stating the fact that a not strictly localized and only slight injury to the brain disposes the central nervous system to react with convulsions after a very small dose

of a convulsant dye. We have thus produced a "*locus minoris resistentiae*," but this phrase, of course, is not an explanation. We can only speculate as to the alterations in the intracerebral mechanism that permits the rapid entrance of the dye stuff into it and increases its disposition to react with convulsions. An increase of the permeability of the brain vessels seems to take place. It might be that inhibitory influences are abolished but not as a consequence of the removal of the nervous tissue in which those influences are supposed to originate. It is also possible that the brain lesion acts as a stimulating process for subcortical centers which control the outgoing motor impulse.

We wish to emphasize again the fact that quite aside from the increased disposition to convulsive reactions an increased absorption of the dye circulating in the blood vessels is caused by the brain injury. The two circumstances work in the same direction, namely, to accelerate the visible physiological reactions (convulsions), but we think there are definite indications which enable us to separate the two conditions and to show that they both follow injury of the brain.

We think that the effect of the injury is a diffuse alteration in the central nervous system, in which we can single out an increased permeability of the blood vessels, an increased readiness to convulsive reaction and a visible functional change (the convulsion).

In this connection reference must be made to a preliminary communication by Thomas (18) published a year ago on some of the factors that influence the production of acid fuchsin convulsions in frogs. This author finds that "Of slight injuries to the brain, a simple pin prick in the region of the optic lobes, or just anterior to them is as effective as decerebration in bringing on convulsions." Thomas concludes that "cerebral inhibition is a minor factor, if it is a factor at all, in determining the time of onset of acid fuchsin convulsions in frogs." It must be evident that we are dealing here with very complicated matters and we shall make no further comment on the findings or conclusions of this author until we can have access to his detailed paper. As far as concerns injury to the optic lobes and the region just anterior

to them my results corroborate his findings, but in several other respects, as for example, in regard to injury of the olfactory lobes which according to him is effective in causing convulsions I do not find myself in agreement with him.

SUMMARY

After injection of acid fuchsin into the dorsal lymph sac of a frog and injury to the brain a marked absorption of the dye takes place followed by convulsions.

The absorbed acid fuchsin appears to be uniformly distributed throughout the entire substance of the brain and cord and in the quantities here employed it is always found to be present in these organs in the form of a colorless compound.

The decolorized acid fuchsin is found to be present in these tissues in so short a time as two minutes after the brain injury; the interval between brain lesion and convulsions may be somewhat longer.

The injury to the brain is considered to be the cause for the increased absorption of the dye.

In frogs with an intact cerebral nervous system we do not find absorption or only to a very slight degree (after injection of the dye in the quantities used above) and these frogs rarely show convulsions.

The occurrence of convulsions can not be explained by the increased absorption alone. Quite apart from this, the brain injury causes an increased disposition of the central nervous system to react with convulsions. A slight absorption of the dye in the intact brain is usually not followed by convulsions. We have no explanation to offer at the moment for this increased readiness of the central nervous system to react toward convulsive drugs of the type here studied but we have come to the conclusion that we are not merely dealing with the removal of inhibitory influences passing from the cortex to the subcortical centers.

The causes for the increase of absorption are not to be found in any alteration in the action of the heart or lymph hearts which results from the brain operation.

The acid fuchsin which comes into contact with the injured region, when blood containing the dye bathes this area does not enter to a marked degree the nervous substance at the injured place and does not spread out from there quickly into the other parts of brain and cord.

The dye which is contained in the spinal fluid (after bleeding has occurred) appears not to be absorbed by the substance of the central nervous system to any considerable extent.

The conclusion is drawn that the brain injury causes an absorption of dye into the nervous substance directly from the blood vessels of the brain. It seems possible that the increased permeability of the blood vessels is caused by a change of the innervation of the blood vessels, but perhaps it is to be explained by a change in the condition of the nervous tissue (widening of perivascular and perineuronal spaces) or, finally the increased absorption of dye stuff may be due to a change in the pressure relations between the blood in the brain vessels and the tissue-fluid in the surrounding nervous substance.

The meninges in frogs protect the brain against the influence of certain crystalloid and colloid solutions brought into contact with their outside surface. Ten per cent sodium chloride solution, solutions of picrotoxin, strychnine nitrate and acid fuchsin put on the outside of the meninges cause convulsions only after the meninges have been cut open. Under these circumstances the acid fuchsin produces convulsions without brain injury because it is applied in high concentration. The convulsions in these animals probably are largely due to a direct influence of the acid fuchsin on the medulla oblongata. The presence of a region (Heubel's center) in the nervous structure of the posterior part of the medulla oblongata which controls motor impulses, is also made probable by the depressive effect of transection below the calamus scriptorius.

The brain injury is effective when done posteriorly to the fossa limbica, i.e., in the most anterior part of the cerebral lobes. Removal of the olfactory lobes is not sufficient. Removal of the optic lobes and of the anterior part of the medulla oblongata is also followed by convulsions. Slight prickings of the cere-

bral lobes, of the optic lobes and of the medulla oblongata have the same effect as the above mentioned removal of the anterior third of the cerebral lobes.

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FIG. 1.

The illustration shows the striking difference in the absorption of convulsant dyes into the intact and into the injured nervous system. In A and B acid fuchsin was used, in C and D phenol-sulphone-phthalein. Figures A and C show the crushed brain and spinal cord of frogs in which, after the injection of the dye, no brain injury was performed. The nervous tissue is not stained in these cases, no absorption of dye into brain and cord took place. Figures B and D give a picture of the central nervous system of animals in which the incorporation of dye was coupled with brain injury (and followed by convulsions). The marked pink color proves an absorption of a considerable amount of the dyes into the nervous tissue.



A



B



C



D

FIG. 1



THE EFFECT OF QUININE INTOXICATION ON THE RESPIRATORY CENTER OF THE RABBIT

H. SUGATA AND A. L. TATUM

From the Laboratory of Pharmacology of the University of Chicago, Chicago

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Recently there has been demonstrated in this laboratory a rise in alkaline reserve capacity of the blood in rabbits following quinine intoxication (1). Since the mechanism of this rise in reserve seemed to be obscure, it appeared highly desirable to further analyze the factors contributing to this effect. It is therefore with such a problem that this paper is concerned.

In table 1 are given the results of a few typical experiments on quinine and its effects on reserve. It is to be noted that whereas at the two hour period of quinine action the reserve capacity has increased, at the three or the four hour period the reserve occasionally fell to a value below normal. The values of pH obtained by the indicator method were unchanged throughout.

In general, alkaline reserve capacity may conceivably be increased by retention of CO_2 by cardiac or respiratory depression providing such depression is of such an order as to avoid oxygen want. By direct observation, animals under the influence of quinine do not show symptoms of either cardiac or respiratory depression since the respiration rate is accelerated and the ear veins well distended with "arterial" blood and in no way indicate asphyxia. Bleeding from the ear vein is free and easy. We fully realized however that the color of blood is no indicator of CO_2 content but only of O_2 saturation.

Blood pressure records were made in urethanized animals and no significant change could be found that could explain the rise of reserve on the basis of cardiac inefficiency.

Artificial respiration, was resorted to in order to determine whether quinine would produce its typical reserve changes when

respiratory ventilation was maintained constant. As may be seen from table 2, no reserve rise occurred during the time of

TABLE 1

The effect of quinine on the alkaline reserve capacity and pH of the blood of the rabbit.*

NUMBER OF ANIMAL	WEIGHT	TIME	ALKALINE RESERVE CAPACITY	pH	NOTES
	kilos.		cc.		
1	1.8	1:40 p.m.	0.71		200 mgm. quinine HCl, intramuscularly
		2:00			
		4:00	0.92		
2	2.2	5:00 p.m.	0.82		250 mgm. quinine HCl intramuscularly
		5:10			
		7:20	0.98		
3	2.4	7:00 a.m.	0.68		250 mgm. quinine HCl intramuscularly
		9:15			
		11:15	0.85		
4	2.8	8:40 a.m.	0.72	7.7	300 mgm. quinine HCl intramuscularly
		9:00			
		9:30	0.71	7.7	
		10:00	0.78		
		10:30	0.81	7.7	
		11:00	0.64		
5	3.0	1:20	0.76	7.6	400 mgm. quinine HCl intramuscularly
		1:40			
		2:30	0.88	7.7	
		3:00	0.72	7.7	
		3:30	0.66		

* Experiments 1, 2, and 3 are typical of many experiments some of which are published in the reference cited.

Experiments 4 and 5 are typical of six experiments.

artificial respiration, though during a period wherein artificial respiration was stopped the reserve rose, to fall on reinstituting artificial respiration.

TABLE 2

*The effect of quinine on the respiratory center of the rabbit**

NUMBER OF ANIMAL	WEIGHT	TIME	ALKALINE RESERVE CAPACITY	EXPIRED AIR VOL- UMEN PER 2 MINUTE PERIOD	NOTES
	<i>kilos.</i>		<i>cc.</i>	<i>cc.</i>	
6	2.8	3:20 p.m.	0.69		Urethane, 1 g/kilo
		3:30			
		4:00	0.72		
		4:30	0.68		
		4:40			Artificial respiration
		5:00	0.56		
		5:15	0.57		300 mgm. quinine HCl
		5:20			
		6:00	0.56		
		6:30	0.56		
		7:30	0.50		
7	2.8	3:40	0.76		Urethane, 1 g/kilo
		4:30	0.84		Artificial respiration
		5:00			
		5:30	0.61		
		5:50	0.60		300 mgm. quinine HCl
		6:00			
		7:30	0.80		Stopped artificial respiration
		8:00			
		8:30	0.59		
8	2.0	1:30	0.76	1550	300 mgm. quinine HCl
		1:31			
		2:25	0.88	1400	
		3:00	0.84	1400	
		3:30	0.69	1250	
9	4.8	9:00	0.70	2400	500 mgm. quinine HCl
		9:01			
		10:00	0.87	2120	
		11:00	0.76	2000	

* Experiments 8 and 9 are typical of eight experiments.

Experiment 10 is typical of six experiments.

Experiments 11 and 12 are typical of five experiments.

Experiment 14 is typical of four experiments.

TABLE 2—Continued

NUMBER OF ANIMAL	WEIGHT	TIME	ALKALINE RESERVE CAPACITY	EXPIRED AIR VOL- UME PER 2 MINUTE PERIOD	NOTES
	<i>kilos.</i>		<i>cc.</i>	<i>cc.</i>	
10	2.0	10:00 a.m.	0.74	1480	Double vagotomy
		10:15			
		4:00 p.m.	0.70	1450	
		5:00	0.71	1400	
11	2.2	9:00	0.69	1650	Three hours after vagotomy 300 mgm quinine HCl
		9:05			
		10:00	0.62	2100	
		10:30	0.60	1830	Animal died
		11:00			
12	2.2	12:00 m.	0.76	1760	Normal
		12:05 p.m.			Double vagotomy
		7:00	0.76	1730	200 mgm. quinine HCl
		7:05			
		7:50	0.74	2050	
		9:00	0.59	2450	Animal dead
		9:30			
13	2.0	3:00 p.m.	0.76	1350	Normal
		3:05			Double vagotomy
		5:00	0.76	1450	20 mgm. morphine
		5:05			
		6:30	0.80	1000	
		7:30	0.82	550	
14	2.4	1:30 p.m.	0.76	1690	After vagotomy 20 mgm. morphine 200 mgm. quinine HCl
		2:20	0.84	1220	
		3:00	0.82	1080	

Such results rather strongly indicated respiratory inefficiency under quinine intoxication in spite of the rapid respiration almost invariably observed. Respiratory inefficiency in instances of rapid respiration could occur providing the depth were diminished

out of proportion to the increased rate. Consequently respiratory volume was determined by a common laboratory method of water displacement.

In experiments 8 and 9, table 2, is clearly illustrated an important fact, namely the occurrence of a distinct and evidently significant diminution of respiratory volume.

Since the vagus nerves normally exert a regulatory influence on the respiratory center particularly in regard to depth, we repeated the experiments on animals previously vagotomized and allowed to recover from the light ether anesthesia required for a few moments for the section.

Simple double vagotomy has no evident effect on the air volume. But after quinine in the usual dosage, air volume was increased quite distinctly with an accompanying fall in reserve capacity of blood (experiments 11 and 12). Furthermore this same usual dosage was nearly always fatal after double vagotomy while quinine alone is invariably recovered from and double vagotomy alone does not cause the death of the animal for many hours.

As is well known, morphine depresses the rate and depth of respiration, particularly the former leading to diminution of air volume. Hence it was considered logical to treat the quinine poisoned double vagotomized animals with morphine and found a remarkable alleviation of symptoms of dyspnea and hyperpnea, a diminution of air volume, absence of acute fall in reserve and recovery from the acute and otherwise fatal action of quinine.

DISCUSSION

The rise in alkaline reserve capacity induced by quinine poisoning is to a very large extent if not completely due to a diminution of respiratory ventilation to an extent as to lead to incomplete removal or retention of CO_2 , without at the same time causing O_2 want. Too great diminution of respiratory volume should cause a degree of oxygen unsaturation with consequent acid production and fall of reserve which latter has been observed.

The diminution of respiratory volume is of especial interest to us inasmuch as there is an increased rate under quinine action.

The depth is evidently so decreased by the increased rate that the volume per unit time is decreased. This strongly resembles the effects produced by electrical stimulation of the central end of the divided vagus nerve. A proper strength of stimulus will accelerate the rate—whereas greater strength is usually found to “choke” the center so that the depth approaches zero, in other words, no evidence of respiratory movements, commonly called inhibition.

By elimination of afferent vagus influences by double vagotomy, the action of quinine is evidently that of increasing the responsivity of the respiratory center to CO_2 with a resulting overactivity of the respiratory mechanism, increased air volume fall in reserve, pulmonary congestion and eventually heart failure—all strongly suggestive of acapnia induced by overactivity of the respiratory center. We have tried to meet this possibility by causing rebreathing hence combating the acapnia but such procedures appeared to aggravate the symptoms. In these circumstances it is conceivable that the excess CO_2 further stimulates the over-irritable respiratory center with subsequent paralysis even though the heart itself may be bettered.

Morphine appears to answer the requirements in that it depresses the respiratory center, prevents excessive loss of CO_2 as judged by the prevention of fall of reserve capacity of blood, and leads to recovery from the acute effects of quinine.

In over-dosages of quinine and frequently later in the course of our usual standard dosage there occurs a depression of the respiratory center following a period of irritation or over-excitability. In these instances the reserve falls, presumably from oxygen want.

It may be stated here for purposes of comparison of quinine with other respiratory stimulants that caffeine in our experience, increases respiratory volume in both normal and double vagotomized rabbits thus differing materially from the action of quinine.

CONCLUSIONS

1. Quinine, in essentially toxic doses when administered to rabbits, causes a rise of alkaline reserve capacity largely if not entirely due to diminution of pulmonary ventilation.

2. The decreased pulmonary ventilation may occur, subsequent to proper dosage, in such an order as to cause retention of CO_2 without O_2 deficiency.

3. In the intact animal respiratory volume is decreased by the increased rate which is too shallow to be normally efficient.

4. In double vagotomy alone, respiratory volume is not essentially changed.

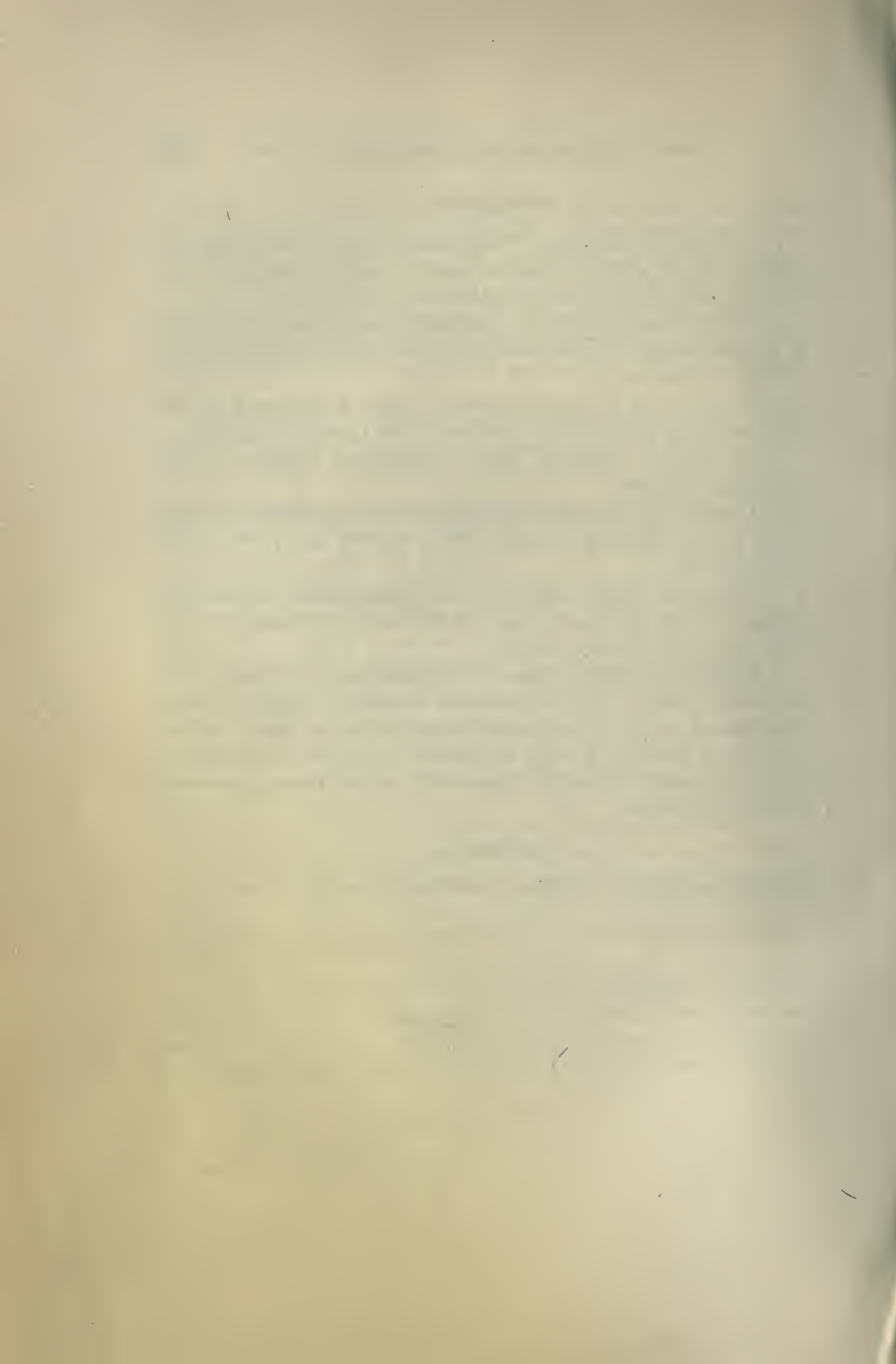
5. Double vagotomy with quinine increases respiratory volume above normal, leading to rapid fall in reserve and early death of the animal.

6. Morphine, double vagotomy, and quinine reduce respiratory volume so that the reserve may not fall and the animal recovers from the acute effects of quinine.

7. Quinine, therefore, appears to increase the irritability of the respiratory center: (a) reflex response to afferent vagus impulses—increasing rate at the expense of depth leading to under-ventilation, (b) in absence of vagal influences the increased irritability of the respiratory center to “hormone” action causes excessive or over-ventilation.

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EXPERIMENTAL RESEARCH ON THE DISTRIBUTION AND ELIMINATION OF ORGANIC ARSENIC COM- POUNDS AFTER INTRAVENOUS ADMINISTRATION

F. M. R. BULMER

Department of Therapeutics, University of Toronto

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In recently reported experiments (1) no evidence was found that arsenic is present in the spinal cord after the intravenous injection of phenarsenamine even in large doses. It was also shown there that the arsenic content of the liver and blood one hour after administration varies with the dose.

This work has led to a study of the distribution of arsenic in certain organs of the rabbit, and to a quantitative examination of the arsenic output in the feces and urine of dogs.

As far as the writer is aware, no systematic investigation of the quantitative distribution of arsenic in the organs following intravenous injection has hitherto been made. Tests have merely shown it to be present in the liver, spleen, long bones and elsewhere. Information on these points is obtainable mainly from toxicological work. Nor has its excretion been followed in a satisfactory manner. Frenkel-Heiden and E. Navassart (2) in 1913 found more arsenic in the feces than in the urine after intravenous injection.

From the contents of a duodenal tube Obregia and Carniol (3) in 1915 obtained a positive test for arsenic and thus concluded that there is arsenic in the bile. This work is, however, inconclusive.

In 1920 Pomaret (4), after making a biliary fistula in a dog, found that after intravenous injection of arsenic, the arsenic was always present in greater amounts in the bile than in the urine.

DISTRIBUTION

No attempt has been made here at an exhaustive study of the organs. Analyses, only of those organs and tissues which in preliminary experiments, seemed to be closely associated

TABLE 1

	TIME KILLED AFTER ARSENIC	ORGANS AN- ALYSED	WEIGHT OF ORGANS	ARSENICAS As_2O_3 IN ORGANS	ARSENICAS As_2O_3 PER GRAM WEIGHT OF ORGAN
			gm.	mgm.	mgm.
Rabbit 1. Weight 2325 grams; dose 8 mgm. phenarsenamine per kilogram.	5 minutes	Femur	10.6	0.015	0.0014
		Kidneys	11.5	0.08	0.007
		Lungs	8.0	0.06	0.0075
		Liver	55.2	1.3	0.0236
		Blood	10 cc.	0.016	
Rabbit 2. Weight 1900 grams; dose 8 mgm. phenarsenamine per kilogram	1 hour	Femur	10.2	0.015	0.0014
		Kidneys	14.2	0.020	0.0014
		Lungs	11.5	0.06	0.0052
		Liver	86.2	0.15	0.0017
		Blood	10 cc.	0.010	
Rabbit 3. Weight 2050 grams; dose 8 mgm. phenarsenamine per kilogram.	1 day	Femur	9.1	0.013	0.0014
		Kidneys	18.5	0.020	0.0010
		Lungs	10.9	0.010	0.0009
		Liver	63.4	0.125	0.0019
		Blood	10 cc.	0.008	
Rabbit 4. Weight 1965 grams; dose 8 mgm. phenarsenamine per kilogram.	4 days	Femur			
		Kidneys	15.42	0.006	0.00038
		Lungs	8.9	0.006	0.00069
		Liver	101.3	0.02	0.00019
		Blood	10 cc.	0.001	
Rabbit 5. Weight 2400 grams; dose 8 mgm. phenarsenamine per kilogram.	10 days	Femur	10.53	0.004	0.00038
		Kidneys	15.00	0.005	0.00038
		Lungs	9.7	0.002	0.0002
		Liver	67.26	0.008	0.0001
		Blood	10 cc.	None	

Phenarsenamine, 30 per cent arsenic.

with the localization of arsenic, were undertaken. In most cases, rabbits were used for the distribution experiments, mainly for the reason that the whole organ or organs could be taken for analysis.

After the intravenous injection of phenarsenamine—in dose relative to that given to man—the arsenic is found chiefly in the liver, lungs, kidneys and long bones. In some cases the lungs contain more arsenic than the liver, especially when larger doses are given. An hour after the injection, the lungs consistently contain more arsenic than does the liver per gram weight of tissue. The preponderance of arsenic in lung tissue persists for at least several days after the injection. It is interesting to note, however, that, when the animal is killed five minutes after the injection has been made, the liver contains more arsenic than the lungs per gram weight. As may be seen later, this rapid disappearance of arsenic from the liver within the first hour, may be accounted for by its elimination in the bile.

TABLE 2

Analysis of organs of dog killed forty days after intravenous injection

Weight, 30½ pounds; dose, 183 mgm. phenarsenamine. Arsenic found in urine and feces for thirty-eight days.

ORGAN	WEIGHT	ARSENIC AS As ₂ O ₃ IN ORGAN	ARSENIC AS As ₂ O ₃ PER 100 GRAMS OF ORGAN
	gm.	mgm.	mgm.
Liver.....	404	0.024	0.006
Lungs.....	80	0.0032	0.004
Heart.....	118	0.002	0.002
Kidneys.....	69	0.0041	0.006
Spleen.....	51	0.001	0.002
Femur.....	46	0.004	0.008
Blood.....	10 cc.	None	

Table 1 shows the results of experiments with 5 rabbits killed at varying intervals. The rabbits received 8 mgm. phenarsenamine per kilogram.

The persistence of arsenic in the femur in relatively high amount is a fact probably of some significance.

Table 2 shows the results of the analyses of certain organs of a dog which weighed 30½ pounds, and had been given intravenously a single dose of 183 mgm. phenarsenamine. This dog was killed forty days later. Arsenic was found in the urine and feces until the thirty-third day.

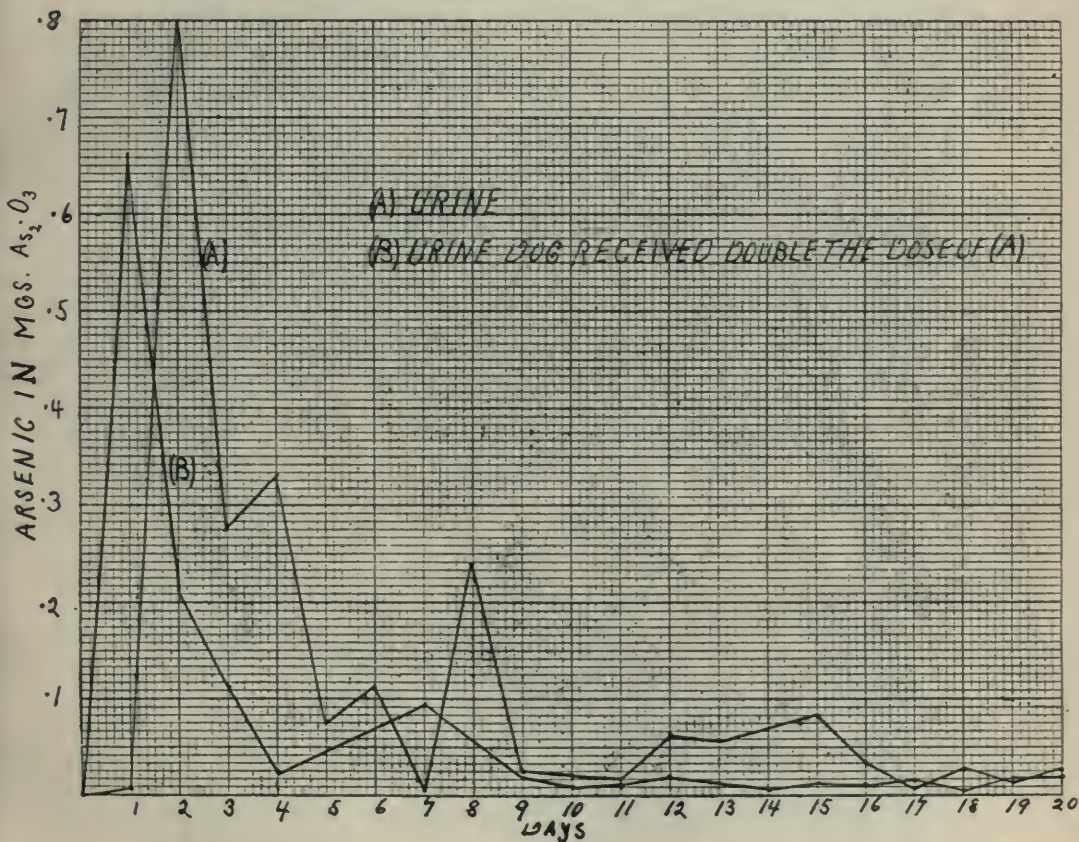
The high arsenic content of the femur in this dog after forty days, is in accord with the retention of arsenic in the femurs of the rabbits in table 1.

TABLE 3

NUMBER OF DAYS AFTER INJECTION	DOG A (WEIGHT 36 POUNDS; DOSE 108 MGM. PHENARSENAMINE)			DOG B (WEIGHT 30½ POUNDS; DOSE 183 MGM. PHENARSENAMINE)		
	Volume of urine	Arsenic in urine as As ₂ O ₃	Arsenic in feces as As ₂ O ₃	Volume of urine	Arsenic in urine as As ₂ O ₃	Arsenic in feces as As ₂ O ₃
	cc.	mgm.	mgm.	cc.	mgm.	mgm.
1	52	0.006		1120	0.67	
2	575	0.805		720	0.216	
3	550	0.275	1.6	1320	0.105	24
4	555	0.333		600	0.024	
5	600	0.078				0.05
6	540	0.129	6.8			0.40
7	30	0.006	2.6	465	0.093	
8	1430	0.243	0.6			
9	240	0.026	1.4	690	0.027	
10	1220	0.024		1110	0.001	0.025
11	470	0.188		200	0.016	
12	535	0.064	0.4	405	0.024	0.10
13	440	0.057	0.6	540	0.010	0.03
14			0.3	430	0.008	
15	734	0.080		525	0.015	0.02
16	640	0.038	0.2	770	0.015	
17	425	0.008		285	0.018	
18	452	0.027		600	0.006	0.02
19	600	0.012	0.3	800	0.016	0.01
20	820	0.032	0.2	920	0.018	0.04
21	640	0.019		500	0.010	0.005
22	460	0.036	0.06	240	0.004	0.005
23	645	0.025	0.01	900	0.009	None
24	415	0.022	0.04	300	0.003	0.0025
25	1280	0.064		620	0.006	
26	355	0.024	0.08	510	0.010	None
27	900	0.036	0.04	620	0.012	None
28	530	0.010		575	0.005	
29	760	0.007		775	0.014	None
30	620	0.012	None	775	None	0.005
31	490	0.009		750	0.007	0.005
32	820	0.016		740	0.007	
33	630	0.006		860	0.017	0.005
34	420	None	0.01	570	None	
35	830	0.008	0.03			
36	420	None	0.015			
37	975	None				
38	625	None				

ELIMINATION

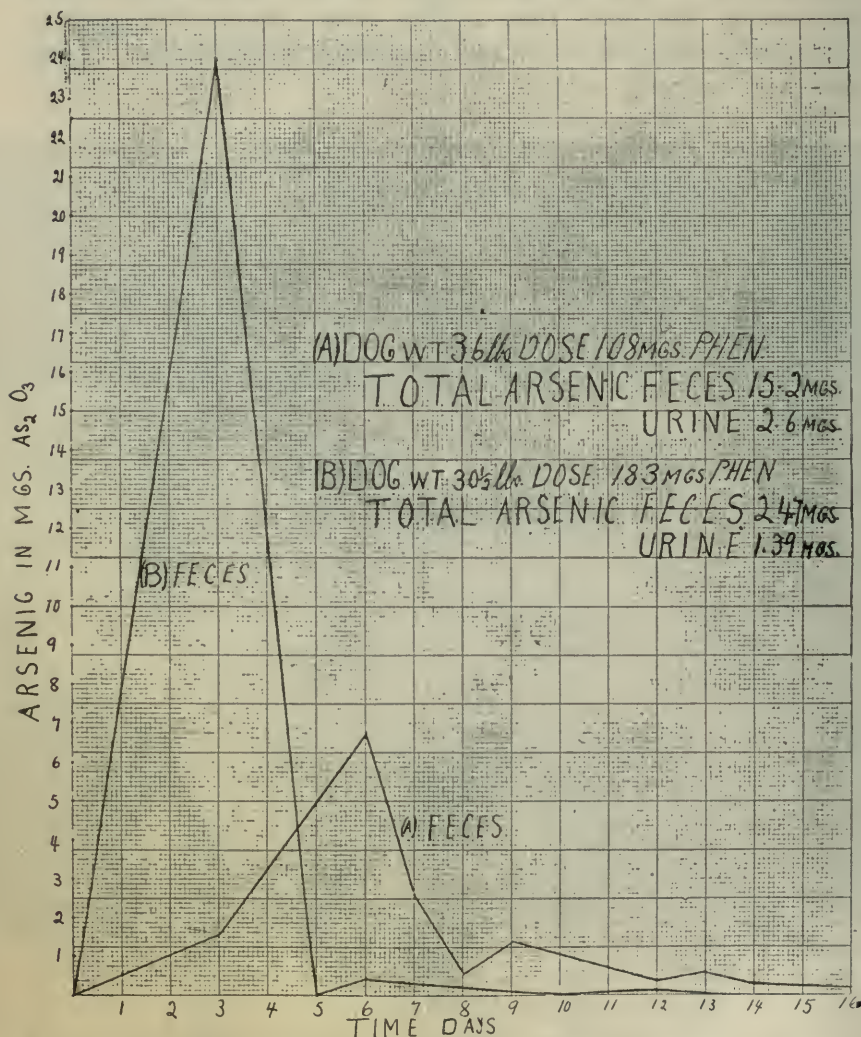
The elimination of arsenic by the feces and urine, was studied in dogs. It soon became apparent that much more arsenic is excreted in the feces than in the urine, though the relative amounts in the two excretions may vary considerably. These facts are shown in table 3.



GRAPH 1

Graph 1 shows the rate of excretion of arsenic in the urine of dogs A and B. Although dog A received only half the dose of dog B, the total amount of arsenic excreted in the urine of dog A, exceeds that excreted in dog B. This unexpected finding may be explained by consideration of graph 2.

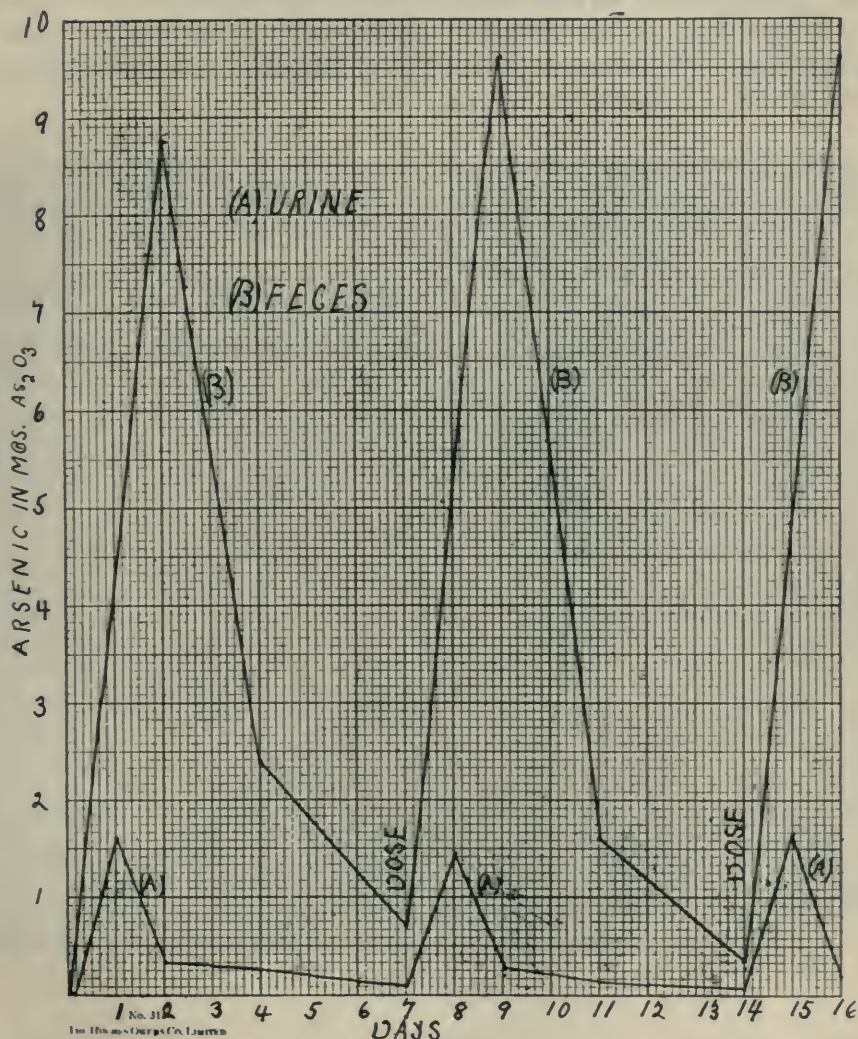
Graph 2 shows the excretion of arsenic in the feces of dogs A and B. It will be observed that the first feces of dog B contained nearly all the arsenic recovered, whereas in dog A, the



GRAPH 2

rate of excretion was much more gradual. The retardation in the excretion of arsenic in dog A may thus account for the higher proportion of arsenic in its urine. The two dogs A and

B represent extreme types. Dog B excreted its arsenic more rapidly than any other dog examined, while dog A represented

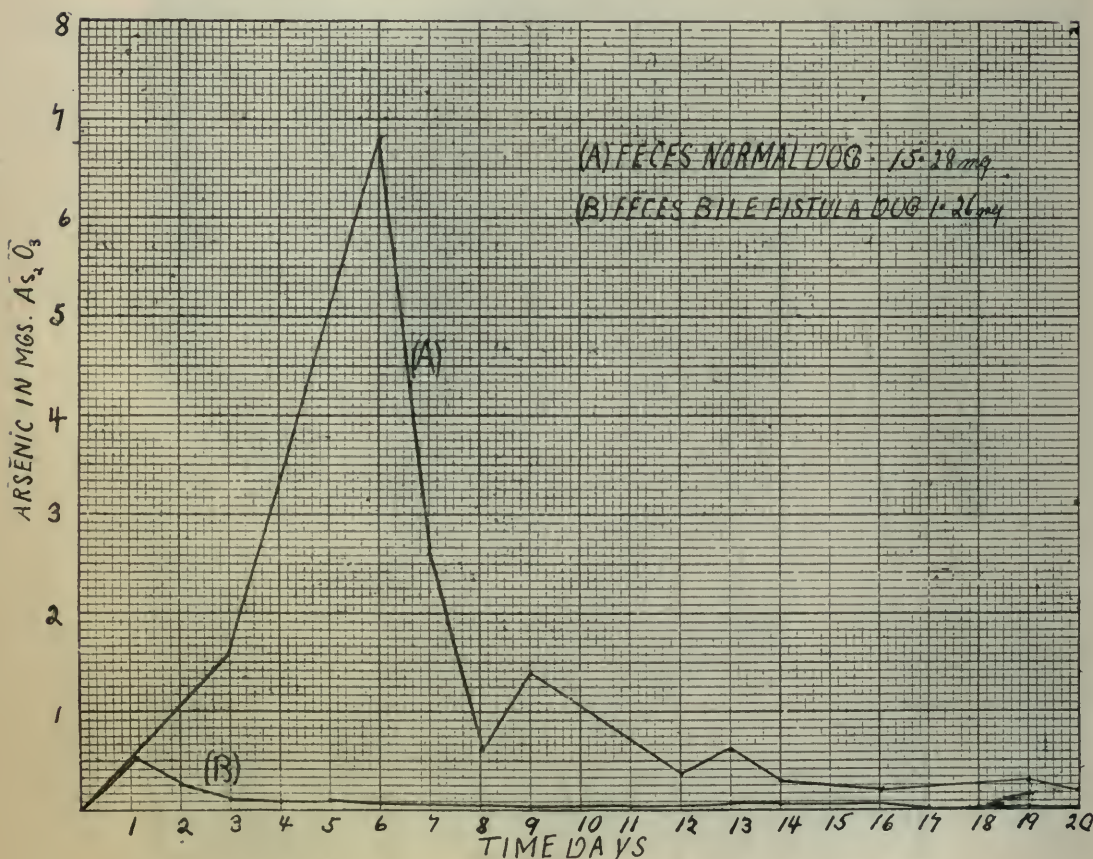


GRAPH 3

the other extreme. Other dogs observed have shown intermediate rates of arsenic elimination.

To determine the effect of successive doses of arsenic on the excretion, a dog was given phenarsenamine in a dose of 4 mgm. per pound every seven days.

Graph 3 shows the results. After each injection, there is a rapid rise in the amount of arsenic in both the feces and urine. Each rise is followed by a rapid fall.

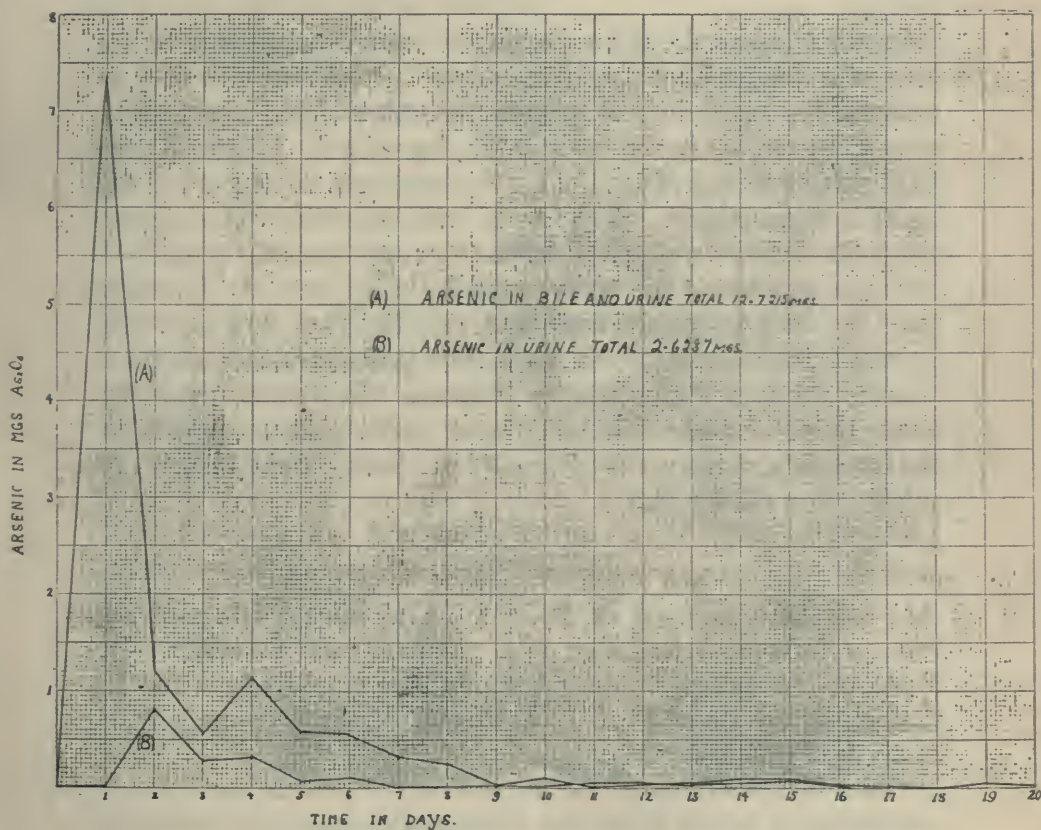


GRAPH 4

The question next arises as to how the arsenic enters the feces. Is the arsenic secreted by the gastro-intestinal tract, or by some external gland that drains into the intestinal canal? The work of Obregia (3) and Pomaret (4) might lead one to

suspect the bile as the vehicle which carries the arsenic into the feces. And, as will be seen below, the bile appears to be the main route by which the organism gets rid of arsenic.

If the arsenic excreted in the feces is primarily from the bile, one should find that, on tying off the common bile duct, and



GRAPH 5

draining the gall bladder, the arsenic should disappear from the feces. The common bile duct of several dogs was thus ligatured, and the feces collected. In all cases, after intravenous injection of phenarsenamine, the arsenic content of the feces was greatly lowered.

On account of the difficulty of collecting the bile separately, it was allowed to drain from the metabolism cage into the urine jar, and the bile and urine were analyzed together. The analyses of the bile and urine showed that the arsenic content was on an approximate level with that found in the feces of other dogs receiving the same dose of arsenic, but which had not been operated on.

Graph 4 shows the difference in the amount of arsenic in the feces of a dog with biliary fistula and a normal dog. Both dogs received the same dose of arsenic per unit weight. Very little arsenic is excreted in the feces of the dog with the biliary fistula. And part of this small amount of arsenic may be accounted for by the contamination of the feces with the bile. The animal also licks a certain amount of bile as it exudes from the wound.

Graph 5 presents the analyses of the urine, and the bile and urine of the dogs represented in graph 4. The urine and bile from the dog with fistula, is marked by its high arsenic content.

From the fact that when the common bile duct is ligatured and a biliary fistula established very little arsenic appears in the feces, it is evident that normally the body eliminates arsenic chiefly through the bile.

DISCUSSION

It may be mentioned at this point, that the various analyses were performed as indicated in a previous communication (1). Much of the success of the technique here employed, is due to the helpful advice of Prof. L. J. Rogers of the Department of Analytical Chemistry.

The salient features in the distribution of arsenic are its high concentration in the liver very shortly after injection; the rapid lowering of this concentration presumably by the elimination of the arsenic by the bile; the relatively large amount in the lungs, especially after large doses, and the maintenance of a high arsenic content here, over a period of at least several days; and its retention by the long bones over a longer period than shown by any other tissue analyses.

The migration of arsenic to the lungs, might suggest an expiratory channel of excretion. This is supported to some extent, by the fact that only about 40 per cent of the arsenic administered can be accounted for in the urine and feces. The suggestion is, however, merely tentative, as there are various other factors to consider before postulating channels of excretion other than the urine and feces.

The retention of arsenic in the long bones is perhaps significant in the view of the therapeutic use of arsenic in anemias.

The work on excretion confirms the findings of Frenkel-Heiden and E. Navassart (2), that more arsenic is excreted in the feces than in the urine. It has been shown here that the arsenic is introduced to the feces by the bile. This important function of the bile in arsenic elimination, has not been hitherto shown in a conclusive manner. The liver rather than the kidney may thus be regarded as the arsenic clearance depot.

The well known fact (5) that liver tissue is impaired by arsenic might in itself suggest that the liver function in arsenic elimination is of some importance. The frequent occurrence of hepatitis following arsenic administration, combined with the established fact that glucose protects the liver from such poisons as phosphorus, has suggested a future line of research whereby it may be determined whether the administration of arsenic in glucose might have less toxic effects on the liver than when it is given in a saline solution.

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The first of these was the fact that the United States had a large and growing population. This was due to a number of factors, including a high birth rate, a low death rate, and a large influx of immigrants from Europe. The second factor was the fact that the United States had a large and growing territory. This was due to a number of factors, including the acquisition of new lands from Spain, France, and Britain, and the discovery of new lands in the West. The third factor was the fact that the United States had a large and growing economy. This was due to a number of factors, including the discovery of new resources, the development of new industries, and the growth of trade with other countries. The fourth factor was the fact that the United States had a large and growing military. This was due to a number of factors, including the development of new weapons, the expansion of the army and navy, and the growth of military spending. The fifth factor was the fact that the United States had a large and growing influence in the world. This was due to a number of factors, including the growth of American power, the expansion of American trade, and the growth of American culture.

The sixth factor was the fact that the United States had a large and growing population of free people. This was due to a number of factors, including the growth of the free labor market, the development of new industries, and the growth of trade with other countries. The seventh factor was the fact that the United States had a large and growing population of free people. This was due to a number of factors, including the growth of the free labor market, the development of new industries, and the growth of trade with other countries. The eighth factor was the fact that the United States had a large and growing population of free people. This was due to a number of factors, including the growth of the free labor market, the development of new industries, and the growth of trade with other countries.

HISTOLOGICAL CHANGES PRODUCED EXPERIMENTALLY IN RABBITS BY BISMUTH

BALDWIN LUCKE AND JOSEPH V. KLAUDER

From the McManes Laboratory of Pathology, University of Pennsylvania, and the Research Institute for Cutaneous Medicine, Philadelphia

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Many different compounds of bismuth have lately been used in the treatment of syphilis, since it has been claimed that in spirocheticidal properties bismuth stands midway between mercury and arsphenamine, and that it may be used advantageously where, for one reason or another, the mercurials and arsenicals are contraindicated. The reports hitherto published have come chiefly from France; most of them deal with sodium and potassium tartro-bismuthate¹ given intramuscularly in aqueous solution, or suspended in olive oil. One of us (J. V. K. (1)) has recently summarized the available literature and reported his own observations on the use of bismuth in experimental syphilis in rabbits, and on the results obtained in the treatment of syphilitic patients in the different stages of the disease.

Now, it is a well known fact that the use of bismuth may give rise to toxic symptoms, due to lesions produced during its elimination from the body, in the oral cavity, the large intestines, the liver, and the kidneys. Among reports by American writers dealing with bismuth poisoning especially from its use in surgery, may be mentioned those of Mayer and Baehr (2), Cabot (3), and Higgins (4).

The present investigation had for its purpose the study of tissue changes produced by such compounds of bismuth as are now used in the treatment of syphilis.

¹ In many of these reports the French trade names "luatol" for the aqueous solution, and "trepol" for the oil suspension of this compound have been employed.

EXPERIMENTAL

A series of adult rabbits were given intramuscular or intravenous injections of the following preparations: sodium and potassium tartro-bismuthate (in aqueous solution and oil suspension) potassium tartro-bismuthate (in aqueous solution) and bismuth trioxide (in oil suspension). To accentuate the effects, amounts somewhat above the maximum tolerated doses were employed (table 1). The drugs were used in the proportion of 0.1 gram to 1 cc. of water or olive oil. Intravenous injections were given into the ear veins, intramuscular injections into the muscles of the thigh. All the animals (excepting no. 22, which was killed) died within sixteen days. They were autopsied soon after death, and sections (formalin fixation, paraffin, hematoxylin-eosin) from the following organs were studied: brain, heart, lung, liver kidney, adrenal, and spleen).

HISTOLOGICAL CHANGES PRODUCED BY BISMUTH

Tissue changes were most evident in the liver and the kidneys. Lesions in other organs were inconspicuous, and consisted mainly of slight cloudy swelling and in the presence within the capillaries of partly conglutinated erythrocytes. The changes in the liver and kidneys are briefly summarized, as follows:

1. *Sodium and potassium tartro-bismuthate*, aqueous solution, intramuscular injection. (Rabbits 49 to 14.) *Kidneys*. The vessels in the cortex are compressed while in the medulla they are slightly engorged. In many, the erythrocytes are conglutinated or fused into hyaline masses. The glomerular tufts are large and almost fill the capsular spaces; they have dark pyknotic nuclei, and in their coils the erythrocytes are usually clumped together or fused into hyaline masses. There is no change in Bowman's capsule or in its lining. The epithelium of the convoluted tubules shows pronounced degeneration in nearly all, and frank necrosis in the majority of the tubules. All intermediate stages between swelling, granularity, delicate vacuolization and definite necrosis are present. The necrotic tubules still have distinct outlines, but their cells are fused into slightly lumpy, almost hyaline masses which completely occlude the tubular lumina so that the affected tubules appear like solid deeply staining cylinders.

TABLE 1

NUMBER OF RABBIT	COMPOUND	ADMINISTERED IN:	ROUTE OF ADMINISTRATION	DOSE PER KILOGRAM	LENGTH OF SURVIVAL
				<i>mgm.</i>	<i>days</i>
49	Sodium and potassium tartro-bismuthate	Aqueous solution	Intramuscular	150	4
28	Sodium and potassium tartro-bismuthate	Aqueous solution	Intramuscular	150	7
53	Sodium and potassium tartro-bismuthate	Aqueous solution	Intramuscular	180	3
14	Sodium and potassium tartro-bismuthate	Aqueous solution	Intramuscular	225	7
36	Sodium and potassium tartro-bismuthate	Aqueous solution	Intravenous	10	1
35	Sodium and potassium tartro-bismuthate	Aqueous solution	Intravenous	15	1
51	Sodium and potassium tartro-bismuthate	Aqueous solution	Intravenous	20	5
52	Sodium and potassium tartro-bismuthate	Aqueous solution	Intravenous	25	4
41	Sodium and potassium tartro-bismuthate	Oil suspension	Intramuscular	200	9
42	Sodium and potassium tartro-bismuthate	Oil suspension	Intramuscular	250	4
43	Potassium tartro-bismuthate	Aqueous solution	Intramuscular	150	12
22	Potassium tartro-bismuthate	Aqueous solution	Intramuscular	250	16
29	Potassium tartro-bismuthate	Aqueous solution	Intramuscular	350	1
44	Potassium tartro-bismuthate	Aqueous solution	Intravenous	10	2
33	Potassium tartro-bismuthate	Aqueous solution	Intravenous	20	7
31	Potassium tartro-bismuthate	Aqueous solution	Intravenous	25	7
24	Potassium tartro-bismuthate	Aqueous solution	Intravenous	30	10
39	Bismuth trioxide	Oil suspension	Intramuscular	450	14
40	Bismuth trioxide	Oil suspension	Intramuscular	500	15

Small quantities of blue staining amorphous dust in the necrotic portions indicate early calcareous deposition. The straight tubules of Henle and the large collecting tubules show relatively little change; they contain, however, hyaline casts and much debris. The lesions are most marked in 2 animals which survived the injection for seven days.

Liver. The cells are poorly outlined, in some areas finely vacuolated, and frequently anuclear. There are occasional small foci of necrosis

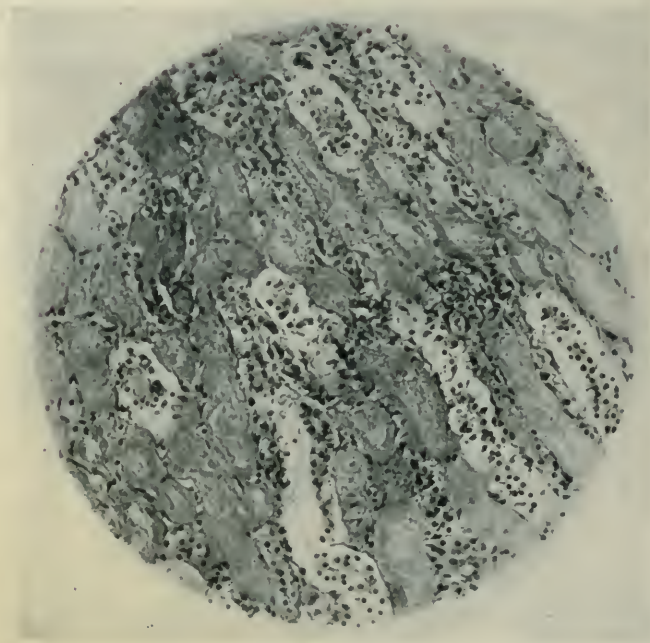


FIG. 1. KIDNEY: NECROSIS OF EPITHELIUM OF CONVOLUTED TUBULES

Rabbit 52; intravenous injection of aqueous solution of sodium and potassium tartro-bismuthate; survived four days.

flooded with poorly preserved erythrocytes, and invaded by small numbers of mononuclear cells and polymorphonuclear leucocytes.

2. *Sodium and potassium tartro-bismuthate*, aqueous solution, intravenous injection. (Rabbits 56 to 52.) In 2 animals surviving the injection only one day the tissue changes are slight. There is moderate cloudy swelling and increased granularity of the epithelium of the convoluted tubules. The glomeruli are large and engorged; their

nuclei are well preserved and the erythrocytes within the loops are clearly outlined. In many of the convoluted as well as in the straight tubules there are hyaline casts. In the animals surviving several days almost all of the convoluted tubules are completely necrotic (fig. 1), and in many portions lime salts are deposited in the dead cells. This is particularly marked in the animal which survived five days, in which nearly the entire cortex is encrusted with calcium.

Liver. There is merely cloudy swelling in 2 animals surviving one day, while in 2 animals living for a longer period many of the cells have lost their nuclei; occasional small foci of necrosis are present.

3. *Sodium and potassium tartro-bismuthate*, oil suspension, intramuscular injection. (Rabbits 41 to 42.) There is extreme necrosis of almost the entire cortex; even the tubular outlines are no longer definite, and many of the glomeruli have disintegrated. A rich lime salt deposit encrusts the dead tubular tissue, the calcification being most marked in the animal surviving nine days. In the medullary tubules the epithelium is often swollen and detached, but no frank necrosis is present and calcification is nowhere seen.

Liver. The hepatic cells are very poorly preserved, very many are anuclear and areas of focal necrosis are numerous.

4. *Potassium tartro-bismuthate*, aqueous solution, intramuscular injection. (Rabbits 43 to 29.) In the animal living for but one day the changes are relatively slight and are characterized by a moderate cloudy swelling of the epithelium of the convoluted tubules; the glomeruli are normal. In the other 2 animals extreme necrosis is present involving almost the entire cortical tissue, excepting the glomeruli which are relatively little altered; indeed in many places they are very well preserved, contrasting sharply with the complete necrosis of the surrounding tubules. A heavy deposition of calcium salts is present in the necrotic regions (fig. 2). Henle's tubules and the collecting tubules contain much debris, but their cells are only here and there swollen and granular; they are in no place necrosed.

Liver. Slight cloudy swelling is present in the animals surviving one day. In the other 2 there are large areas of necrosis flooded with disorganized erythrocytes and invaded by a few mononuclear cells and polymorphonuclear leucocytes.

5. *Potassium tartro-bismuthate*, aqueous solution, intravenous injection. (Rabbits 44 to 24.) In the animals surviving two days the capsular spaces contain much finely granular eosin-staining precipitate; the tufts are compressed. The nuclei of the latter appear slightly

prominent. The capillary coils contain partly clumped erythrocytes. The changes in the convoluted tubules vary from moderately advanced cloudy swelling to early frank necrosis. In the straight tubules there are hyaline casts and much detritus. In the animals surviving longer periods the lesions are more pronounced. The capsular spaces contain relatively little foreign substance, but the convoluted tubules are

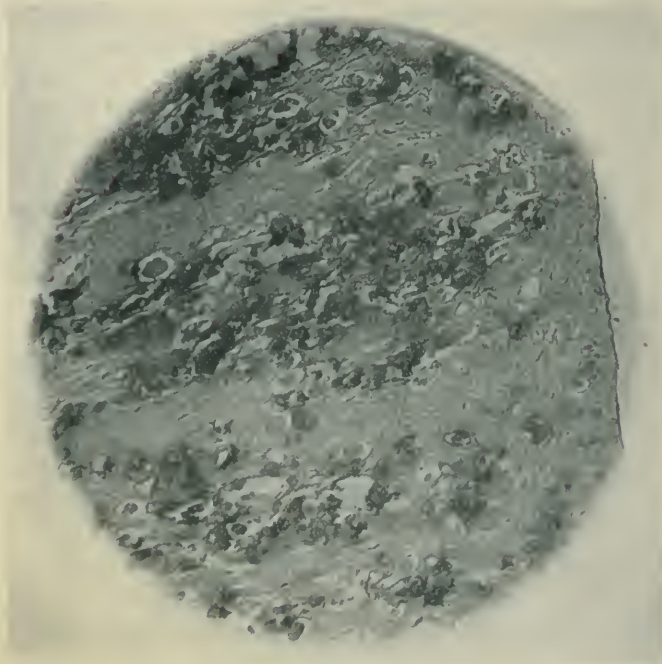


FIG. 2. KIDNEY: NECROSIS OF MANY CONVOLUTED TUBULES, AND ENCRUSTATION WITH CALCIUM SALTS OF NECROTIC PORTIONS

Rabbit 43; intramuscular injection of aqueous solution of potassium tartro-bismuthate; survived twelve days.

more frankly necrotic and in many portions calcareous deposits are present (fig. 3). In the liver there is marked cloudy swelling, with occasional small areas of focal necrosis.

6. *Bismuth trioxide*, oil suspension, intramuscular injection. (Rabbits 49 to 40.) *Kidneys*. There is extreme necrosis and calcification throughout the entire cortex (fig. 4). Many of the glomeruli are disorganized, but others are well preserved, even in necrotic areas. The

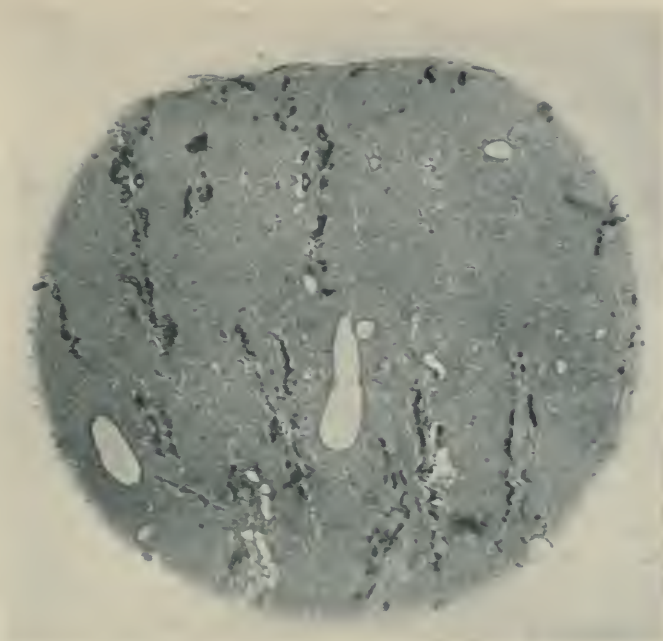


FIG. 3. KIDNEY: NECROSIS AND CALCIFICATION OF MANY CONVOLUTED TUBULES

Rabbit 24; intravenous injection of aqueous solution of potassium tartro-bismuthate; survived ten days.

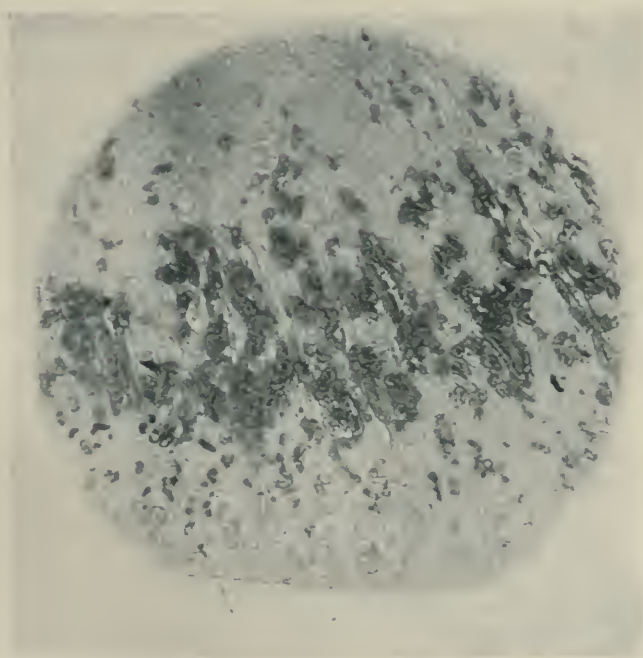


FIG. 4. KIDNEY: MARKED NECROSIS AND CALCIFICATION OF CONVOLUTED TUBULES

Rabbit 39; intramuscular injection of bismuth dioxide; survived fourteen days.

straight and collecting tubules show relatively little change, but very occasionally there is a necrotic and even calcified limb of Henle.

Liver. There is very marked cloudy swelling; occasional small areas of necrosis flooded with erythrocytes are encountered.

SUMMARY AND CONCLUSIONS

The histologic changes produced in rabbits by several compounds of bismuth employed in the treatment of human syphilis are reported. Lethal doses of the drug, in aqueous solution or oil suspension were administered. Injections were made intramuscularly as well as intravenously. All died within sixteen days after poisoning. The following organs were studied histologically: brain, heart, lungs, spleen, adrenals, kidneys, and liver (the gastro-intestinal tract was not studied). Lesions of similar character were constantly found in the kidneys and the liver, while in the other organs, examined the tissue changes were inconstant and inconspicuous. There was considerable variation in the degree of severity depending mainly upon the length of survival of the animals. In general the tissue changes were most pronounced in animals surviving the injection four or more days. The difference in route of administration or in the compound of bismuth injected did not produce essential differences in the character of the histologic lesions, so that the injuries found may be ascribed to the toxic action of bismuth rather than to any one compound or method of administration.

The renal injuries involved chiefly the epithelium of the convoluted tubules. These showed all types of degeneration, ranging from severe cloudy swelling to extreme necrosis and marked calcification. The glomeruli did not appear primarily damaged, and often were found to be well preserved in the midst of completely necrotic tubules. The glomerular capillaries frequently contained partly conglutinated and sometimes hyalinized masses of erythrocytes. The glomerular spaces were usually free from foreign material.

The lesions in the liver were much less conspicuous. There was usually present a definite cloudy swelling, with evident nuclear degeneration, and the appearance of minute fat droplets.

Occasional small foci of necrosis flooded with erythrocytes and invaded by a few mononuclear and polynuclear leucocytes were found in most animals.

It has been shown by one of us (B. L. in collaboration with Kolmer (5)) that in arsphenamine poisoning there are tissue changes in the kidneys and the liver. A comparison of these lesions with the injuries observed in bismuth poisoning discloses the fact that the kidney changes are somewhat more marked in bismuth intoxication, while the liver lesions are more pronounced in arsphenamine poisoning. In mercurial poisoning the renal lesions are almost identical with those caused by bismuth, and in its toxic properties bismuth resembles mercury rather than arsphenamine. The two former substances exert a greater nephrotoxic action, whereas arsphenamine is more hepatotoxic.

Since this work was completed there appeared an article by Kollert, Strasser, and Rosner (6) in which are presented clinical data and urinary findings of patients treated with trepol. The authors also briefly summarize the renal lesions observed in rabbits poisoned with this compound (the details of the work are to be published at a later date). From the description given it seems that the kidney injuries they observed are quite identical with those we have described in this paper.

It may be concluded that caution should be used in the administration of bismuth compounds to patients suffering from renal disease.

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BARBITAL NARCOSIS AND HYPOTHERMIA IN PIGEONS

MAX M. ELLIS

*From the Department of Physiology and Pharmacology, University of Missouri,
Columbia, Missouri*

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INTRODUCTION

As recent work has shown that barbitol can be used satisfactorily as a laboratory anesthetic for dogs over a period of eight to twenty-four hours (Tatum and Parsons (10)) is seemed possible that this drug would prove a suitable narcotic for pigeons in experiments requiring birds which could be held in a quiet and relaxed condition for a day or more. The absence however of literature concerning the effects of barbitol on birds made experiments necessary to determine its specific action on pigeons. The data concerning the general responses of pigeons to barbitol, its action on their body temperature and the dosage of this drug for these birds are given below.

Barbitol unaided did not give satisfactory surgical anesthesia in pigeons. The birds remained rather sensitive to dermal stimulation and the corneal reflex persisted even when large doses were administered, but with proper dosage a very uniform condition of rather deep narcosis at a depressed and fairly constant body temperature level was maintained for hours or days.

MATERIAL

The experimental birds were common pigeons, weighing from 300 to 400 grams. These pigeons had lived for over three months in a large loft and fly-way, and during that time had been fed a standard diet of hard grain supplemented with ample quantities of salt, reddle and grit. Birds selected for tests were given no food in the twelve hours preceding the experiments, and while

under observation were kept in small wire cages together with several control pigeons, in a steam heated laboratory. These controls, it may be added, were apparently in as good condition after eight weeks of uninterrupted confinement in the laboratory cages as when brought in from the loft, as they had not lost weight and were active and alert.

Crystalline barbital (diethylbarbituric acid) of Merck dissolved in a 1-per cent solution of sodium carbonate was the reagent used in all barbital tests. Thirty milligrams of barbital were added to each cubic centimeter of carbonate solution and the mixture heated almost to the boiling point. This concentration of barbital (30 mgm. per cubic centimeter) was chosen as all of the barbital remained in solution after the fluid had cooled, while needles of barbital reappeared at room temperature in a slightly higher concentration (35 mgm. per cubic centimeter).

The barbital solution was injected either into the crop or the abdominal cavity of the pigeon. The crop injections were made through a small rubber tube which was passed down the throat of the bird into the crop. There was however too large a factor of error in this method for definite determinations, as partial regurgitation frequently followed the removal of the tube from the crop. There also seemed to be considerable variation in the rate and completeness of the absorption of barbital from the crops of different pigeons. Cushny (3) states that morphine seems to be absorbed with difficulty from the crops of pigeons and this is apparently true of barbital. Pigeons given equal amounts of barbital on the per kilo of body weight basis, by crop, succumbed in from fifteen minutes to two hours and a half, and there were even greater variations in the duration of the narcosis produced. Accordingly the results from only four crop injections of barbital (not followed by regurgitation) have been included in the dosage table.

The intra-abdominal injections were made at a point just below the end of the breast bone, with a small Luer syringe, the needle of which was directed toward the head of the bird and barely pierced the abdominal wall. In this way the solution was introduced into the abdominal cavity without injury to the

internal organs. To avoid shock the fluid to be injected was always heated to approximately the temperature of the bird. Such injections of barbital, in contrast to the crop injections, gave very prompt and uniform results. The birds recovering from the non-fatal doses showed no after effects from the injection itself.

GENERAL EFFECTS

In general the reactions of the pigeon to barbital were of the same character as those described for mammals. The bird experienced a marked fall in body temperature, frequently developed convulsive movements and opisthotonus for a few hours, and passed into a state of rather uniform narcosis. The state of narcosis was conspicuous in the pigeon experiments by its uniformity and long duration. Developed during the first three hours after the injection of the drug, the narcosis continued with little change for two to twelve days, yet terminating in many cases in a rapid recovery. This prolonged narcosis was preceded by two rather well defined stages, especially if the body temperature were considered, and followed by a fourth stage in which there was either rapid recovery or death.

First stage. Shortly after the abdominal injection of barbital the pigeon showed drowsiness and loss of fear. The attitude changed to that of a well-fed pigeon dozing on its perch. The bird's feathers were slightly raised, its head drawn in close to the body, and its eyes opened and closed lazily. When in this state the pigeon could be handled with little or no struggling on the part of the bird, no matter how wild and excitable it had been just previous to the injection. Removed from its cage the bird stood quietly, showing no particular interest in its surroundings. All of these changes just described came over the bird suddenly during the first ten or fifteen minutes after the injection of from 100 to 350 mgm. of barbital per kilo of body weight.

Gradually the dozing gave way to a deeper sleep in which the eyes were closed most of the time. The bird became more and more unsteady, developing a rocking motion of the body, and losing its balance finally fell. The stimulations resulting from the

fall frequently awakened the pigeon if the dose of barbital were small, and the bird would struggle to its feet and fall again several times before completely losing its ability to stand. If the dose were 200 mgm. or more per kilo the bird usually fell but once and was unable to rise. As may be seen in the tables all of the pigeons tested, excepting B 14 which received but 100 mgm. per kilo, were unable to stand in less than forty-five minutes after the injection and many collapsed in less than thirty minutes. When unable to stand the pigeon lay on its breast with the head and neck extended so that the bill rested on the floor of the cage. The wings gradually relaxed and drooped away from the body, and the feet were drawn back under the tail with their volar surfaces up.

Second stage. During the first two hours after the birds became prostrate many developed twitchings, convulsive movements and even opisthotonic spasms. Conditions similar to these have been recorded in observations on rabbits, dogs and cats during barbital narcosis (Roemer (7)). In the present experiments on pigeons it was not possible to correlate the severity or the duration of these muscular movements with the size of the dose. In some cases only individual feathers were moved and only slight tremors passed over the body, while birds B 15 and B 6 which were given 150 and 500 mgm. per kilo respectively, both became distinctly opisthotonic. Of 19 cases which were followed particularly for this point 11 developed definite tremors and convulsive movements, and 6 became opisthotonic. The onset and duration of these convulsive movements were also subject to much variation. In general these movements disappeared in the first eight hours of narcosis.

Third stage. After the convulsive movements ceased the pigeon lay in a relaxed condition for hours at a time. In some cases there were slight recurrences of the muscular tremors at irregular intervals, but most of the birds if undisturbed moved very little after the eighteenth hour of narcosis, except in response to the defecation stimulus. Defecation continued at rather regular intervals throughout the entire period of barbital narcosis and the cloacal sphincter maintained good tone, even in those birds

receiving large doses, until shortly before death. Some minutes before defecation the bird became restless and shifted from side to side, although unable to stand. The muscular movements of the body ceased almost immediately after the opening of the cloaca. The duration of this third stage was in general correlated with the amount of barbital given, the length of the period increasing as the dose was increased up to about 300 mgm. per kilo, although the first two stages were of about the same length in all tests regardless of the dose.

Fourth stage. The approach of the end of the period of narcosis could be detected in many birds by the body position. Those about to recover began to move their feet, carrying them forward under the body in the normal perching position so that the bird instead of lying on its breast, crouched on its feet. In this position it continued to sleep for some time, but usually rose to a standing position in the next twelve to eighteen hours. Once standing again recovery proceeded very rapidly. The standing bird opened its eyes, became increasingly more responsive to sounds and began to walk about the cage. Although food and water were kept before these recovering birds they made no effort to take either for several hours after they were first able to stand. A standing bird in this condition however could usually be induced to drink by holding its bill under water for a second or two, after which it would drink voluntarily. As soon as the bird was able to drink water its recovery was usually assured in the next thirty-six hours. The return of fear marked the end of the drug action as far as external signs were concerned, for even after the bird was able to stand and take food it remained more or less indifferent to its surroundings and to handling, in some cases for twenty-four hours or more.

Birds about to die from barbital poisoning were usually recognized by the drooping of the lower eye-lids so that the eyes remained partly open, and by the complete relaxation of the muscles of the neck, which allowed the head to fall to one side. Even when this moribund condition was reached the corneal reflex was usually evident.

TEMPERATURE RESPONSES

The body temperature was obtained by means of a properly standardized rectal thermometer inserted through the cloaca into the terminal portion of the rectum. All readings are given in degrees Centigrade. As Simpson and Galbraith (8) have shown that the body temperature of pigeons follows a rather regular diurnal cycle, the injections of barbital were made between

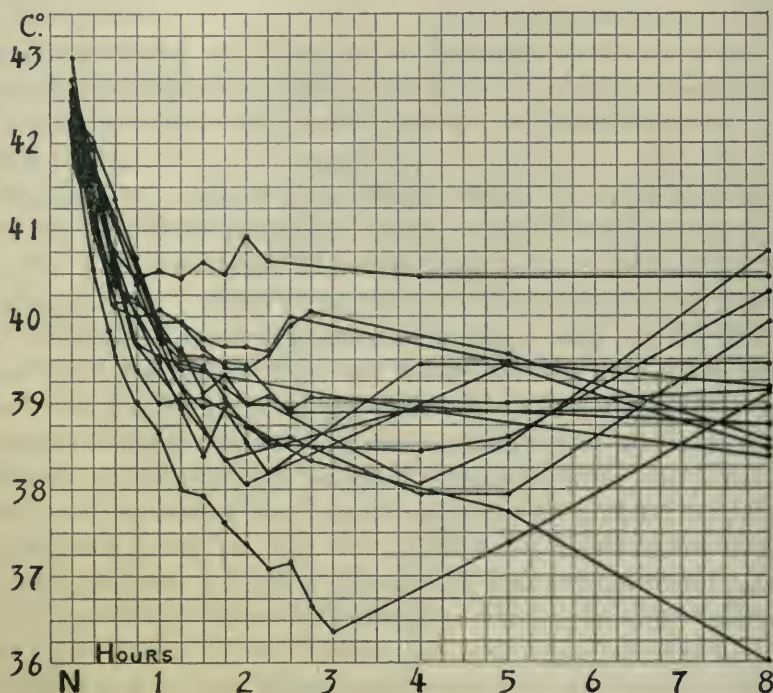


CHART 1. MASSED TEMPERATURE RESPONSES OF 14 ADULT PIGEONS TO DOSES OF BARBITAL VARYING FROM 100 TO 343 MG. PER KILO OF BODY WEIGHT

8:00 and 9:00 a.m. during the period in which the body temperature of the bird was normally rising, so that any immediate depression of body temperature could be ascribed definitely to the drug. The daily temperature readings were taken between 4:30 and 5:30 p.m. as at that time the body temperatures of the male and female pigeons are more nearly the same than at any other time in the day, and the evening decline in body temperature has not yet started.

In chart 1 the temperature responses of 14 adult pigeons to doses of barbitol varying from 100 to 343 mgm. per kilo of body weight are plotted in their proper time relations (for complete data see tables 1 and 2). From this chart it may be seen that the body temperature of each bird was lowered materially in a few minutes after the barbitol was injected, as the body temperatures of all birds regardless of the size of the dose received fell to 40.60°C . or lower in the first forty-five minutes following the injection. The average depression was 0.8° during the first fifteen minutes, 1.7° during the first thirty minutes and 2.8°C . during the first hour. This lowering of the body temperature continued during the second and part of the third hours, so that the minimum temperature in this phase was registered usually about two and one-half hours after the injection, the average depression at that time being about 3.8°C . The actual depression varied in general with the amount of barbitol given, and to some extent with the individual bird, but the minimum body temperature recorded during the first three hours for any pigeon surviving the barbitol injection for eight hours or more was 36.35°C .

During the next five hours, i.e., the third to eighth, the average body temperature rose to 39°C ., about one-half of the cases making slight gains in body temperature and a few conspicuous returns toward the normal. Among those pigeons surviving at the eighth hour only one had suffered a marked fall in body temperature between the third and eighth hours, and that bird (B 11) died on the third day.

After the first eight hours of barbitol narcosis the duration of narcosis and the actual body temperature of the bird varied with the amount of barbitol given. In some cases there was a slow fall in body temperature for twenty-four to forty-eight hours or even longer. After the minimum temperature was finally reached the body temperature in all pigeons making a recovery gradually rose again to between 40.25° and 40.50°C . When this level was reached the bird regained its ability to stand and the body temperature generally rose more rapidly through the next two degrees to the normal of 42.25°C . This correlation between body temperature level and the condition of the bird was a rather definite one throughout the experiments, as the pigeons usually lost their

Changes in body temperature d

PIGEON NUM- BER	NUMBER OF DOSE	BARBITAL	INITIAL TEM- PERATURE	FIRST HOUR AFTER INJECTION				SECOND HOUR		
				15 minutes	30 minutes	45 minutes	60 minutes	75 minutes	90 minutes	105 m

Ad

		mgm. per kilo								
B 14	1st	100	42.35	41.45 S	40.75 S	40.45 S	40.55 S	40.45 L	40.65 S	40.5
B 15	1st	150	43.00	41.45 P	40.10 P	40.00 P	39.45 P	38.90 PC	38.35 PC	38.9
B 10	1st	200	42.35	42.00 S	41.35 S	40.60 P	39.90 PC	39.90 PC	39.75 PC	39.6
B 10	2d	200	42.65	41.75 S	41.05 S	40.45 P	40.00 P	40.10 P	39.90 P	39.6
B 5	3d	225	42.75	41.90 S	40.65 P	39.65 P	39.55 P	39.45 P	39.35 P	39.2
B 8	2d	225	42.20	40.55 S	39.55 P	39.00 P	38.65 P	38.00 P	37.90 P	37.6
B 5	2d	225	42.20	P						38.3
B 12	1st	225	42.35	41.75 S	40.90 L	40.10 P	39.90 P	39.55 P	39.55 P	39.4
B 6	2d	225	42.35	S	P	P	P	39.45 P		
B 2	5th	225	42.10	S	S	P	P			
B 11	1st	225	42.20	41.10 L	40.45 P	39.35 P	39.00 P	39.05 P	39.05 P	
B 17	1st	275	42.75	41.70 PC	41.05 PC	40.45 PC	39.75 PC	39.20 PC	39.00 PC	39.0
B 16	1st	300	42.45	41.35 S	40.55 P	40.20 P	39.75 P	39.65 PC	39.15 PC	39.3
B 18	1st	343	42.45	41.10 L	40.55 P	39.75 P	39.55 P	39.40 P	39.05 P	38.7
B 19	1st	400	42.45	40.55 P	38.90 P	D				
B 12	2d	400	42.20	40.75 P	39.55 PC	PC	38.35 PC	D		
B 20	1st	500	42.55	41.90 P	D					
B 6	3d	500	42.35	41.35 P	PC	39.55 PC	38.35 PC	D		
B 21	1st	600	42.35	41.90 P	D					

S 1	1st	225	42.35	41.20 L	40.00 P	40.00 P	40.20 P	40.35 P	40.20 P	40.5
S 2	1st	225	42.10	41.45 S	40.65 P	39.45 P	39.55 P	39.90 P	40.20 P	40.4
S 3	1st	225	41.20	41.10 L	40.55 P	40.10 P	39.55 P	39.55 P		38.5
S 4	1st	225	41.00	40.75 L	40.00 P	39.45 P	38.90 P		35.90 P	

C, convulsive movements; D, dead; L, lying, but can stand; P, prostrate, cannot stand;

ability to stand when the body temperature had fallen, following the injection of barbitol, to about 40.50°C., and during recovery made their first efforts to stand again when the temperature had returned to about 40.25°C. Similarly, the prostrate condition of maximum narcosis was almost always accompanied by a body temperature of 39° to 39.50°C.

1
at eight hours after barbitol injection

0 minutes	THIRD HOUR				FOURTH HOUR	FIFTH HOUR	EIGHTH HOUR
	135 minutes	150 minutes	165 minutes	180 minutes			
0.90 S	40.65 L				40.45 L		40.45 L
8.55 PC	38.20 PC				39.45 PC		39.45 PC
9.65 PC	39.60 PC	40.00 PC				39.45 P	39.20 P
0.40 P	39.35 P	39.55 PC	39.90 PC	40.10 PC		39.55 PC	38.55 PC
9.00 P	39.10 P	38.90 P	39.10 P			39.00 P	39.20 P
7.35 P	37.15 P	37.20 P	36.65 P	36.35 P		37.35 PC	39.20 P
					38.90 P		38.75 P
9.40 PC		38.90 P					38.90 P
					38.90 P		38.35 P
8.10 P			38.40 P			39.45 PC	38.40 PC
						37.75 P	35.65 P
8.55 PC	38.55 PC				38.45 P	38.65 P	40.55 P
0.00 PC	39.00 PC				38.10 P	38.55 P	40.75 P
8.55 P	38.60 P				37.90 PC	37.90 PC	39.90 P

0.55 P	40.20 P	40.35 P		40.20 P	40.00 P	38.90 P	38.90 P
0.20 P	39.95 P	39.75 P		39.65 P	39.65 P	39.45 P	39.45 P
							38.55 P
5.75 P							35.75 P

S, standing.

Those pigeons which did not show a gradual return to the normal body temperature, following fatal doses of barbitol, did not as might be expected, show a slow decline in body temperature as the narcosis continued. On the contrary they maintained a fairly constant body temperature between 39° and 39.75°C. until death (see barbitol cases in chart 2).

Daily temperatures of

PIGEON NUM- BER	NUMBER OF DOSE	BARBITAL	INITIAL TEM- PERATURE	EIGHTH HOUR	SECOND DAY	THIRD DAY	FOURTH DAY	FIFTH DAY	SIXTH DAY
A									
		<i>mm. per kilogram</i>							
B 14	1st	100	42.35	40.45 L	41.55 S	41.65 S	R		
B 15	1st	150	43.00	39.45 PC	39.75 P	39.10 P	39.65 P	39.70 P	40.00 P
B 10	1st	200	42.35	39.20 P	39.55 P	40.75 S	41.60 S	42.35 R	
B 10	2d	200	42.65	38.55 PC	40.55 S	40.45 S	42.20 R		
B 5	3d	225	42.75	39.20 P	41.10 S	41.00 S	41.35 S	R	
B 8	2d	225	42.20	39.20 P	40.75 S	40.75 S	41.35 S	R	
B 5	2d	225	42.20	38.75 P	38.60 P	39.65 P	39.65 P	39.75 L	40.00 P
B 8	1st	225	43.00	P	P	P	P	38.55 P	40.55 P
B 12	1st	225	42.35	38.90 P	38.35 P	39.55 P	39.45 P	39.75 P	39.35 P
B 6	2d	225	42.35	38.35 P	40.00 P	40.00 P	40.00 P	40.45 L	40.55 P
B 7	1st	225	42.20	P	P	P	P	39.65 P	39.90 P
B 2	5th	225	42.10	38.40 PC	39.00 P	39.90 P	38.90 P	38.45 P	40.00 P
B 11	1st	225	42.20	35.65 P	35.65 P	D			
B 17	1st	275	43.00	40.55 P	40.20 P	D			
B 16	1st	300	42.45	40.75 P	40.45 P	40.00 P	39.90 P	39.70 P	39.75 P
B 18	1st	343	42.45	39.90 P	39.65 P	40.65 P	39.75 P	39.55 P	39.55 P
B									
S 1	1st	225	42.35	38.90 P	41.20 S	R			
S 2	1st	225	42.10	39.45 P	41.45 S	R			
S 3	1st	225	41.20	38.55 P	40.90 S	R			
S 4	1st	225	41.00	35.75 P	40.85 S	R			

C, convulsive movements; D, dead; L, lying, but can stand; P, prostrate, cannot stand.

The changes in body temperature in the pigeon resulting from the injection of barbital, although showing the same general phases of depression and return to normal, differ from those described for mammals both in degree and duration. The maximum depression of body temperature during the first stages of barbital action was reached more rapidly in the pigeon (after about two and one-half hours) than in mammals. Kleist (6) states that the maximum depression of body temperature in

sion on the contrary was less in the pigeons tested than has been recorded for cats and rabbits. Jacobi and Roemer (5) report a

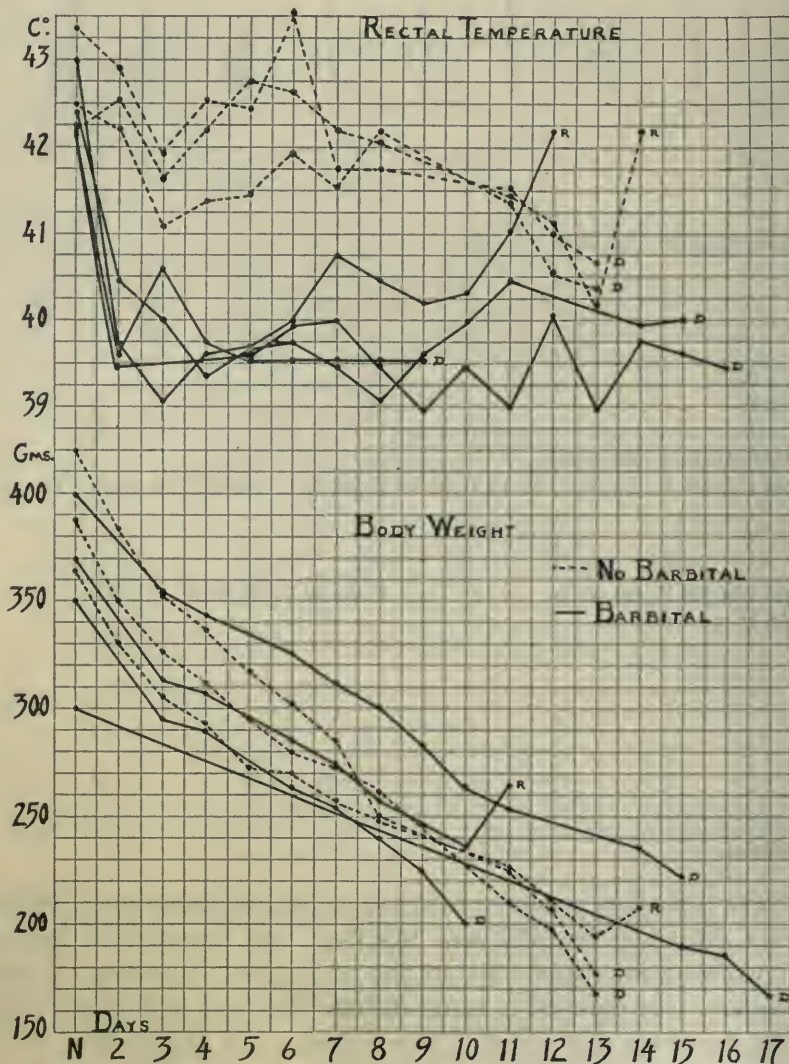


CHART 2. DAILY BODY TEMPERATURES AND BODY WEIGHTS OF PIGEONS IN BARBITAL NARCOSIS COMPARED WITH THOSE OF CONTROL PIGEONS

No food or water given to birds of either series. D, dead; R, recovered.

fall of 10°C . in the body temperature of a cat five hours after receiving 400 mgm. of barbital per kilo. This animal died seven

hours after the injection. The lowest body temperature observed for any pigeon was but 6.55° below its normal, in a pigeon which lived for two days after receiving 225 mgm. of barbital per kilo. The maximum depression recorded from any pigeon injected with 400 mgm., or more barbital per kilo was 4° . Jacobi and Roemer (5) also report a drop of 6.2° in the body temperature of a rabbit following a dose of 400 mgm. per kilo, from which the rabbit recovered in twenty-five hours, as compared with the maximum depression of 5.85° for any pigeon recovering from barbital.

The most conspicuous feature of the pigeon's response to barbital was the slow return to the normal body temperature and the corresponding prolongation of the period of narcosis. In addition to the rabbit case given above, which recovered from 400 mgm. per kilo in twenty-five hours, Jacobi and Roemer (5) state that a cat regained approximately normal body temperature after a depression of 2° as the result of an injection of 300 mgm. per kilo, in ten hours; Berent (2) reports that a dog given 400 mgm. per kilo recovered in two days; and Kleist (6) that a rabbit recovered from 270 mgm. per kilo in twenty-four hours. In contrast to these rapid recoveries in mammals, are 3 cases of pigeons which were in a state of narcosis for ten to twelve days, 6 for five to nine days and 6 for two to four days, produced by doses of less than 250 mgm. of barbital per kilo. These 15 birds recovered. Among those receiving fatal doses of barbital were 3 other birds which remained in deep barbital narcosis for ten to seventeen days before death. Bachem (1) from work on mammals has stated that 50 to 90 per cent of the barbital taken into the body is eliminated through the kidneys, and if this be true for pigeons the prolonged narcosis, which is seemingly indicative of the continued presence of the drug, suggests less efficient elimination by the mesonephritic kidneys of birds.

As the maintenance of body temperature is directly connected with the gross metabolism of the animal and as the birds took neither food nor water during these prolonged barbital narcoses, the correlation between body weight and body temperature was followed in a series of barbitalized and control birds. In this

TABLE 3
Starvation series

PIGEON NUMBER	INITIAL RECORD	SECOND DAY	THIRD DAY	FOURTH DAY	FIFTH DAY	SIXTH DAY	SEVENTH DAY	EIGHTH DAY	NINTH DAY	TENTH DAY	ELEVENTH DAY	TWELFTH DAY	THIRTEENTH DAY	FOURTEENTH DAY	FIFTEENTH DAY	SIXTEENTH DAY	SEVENTEENTH DAY
N 1 {	Temperature 43.35	42.90	41.90	42.55	42.45	43.55	41.75	41.75			41.55	41.00	39.60				
	Weight 420	384	352	336	317	302	285	250			225	208	177 D				
N 2 {	Temperature 42.50	42.20	41.10	41.35	41.45	41.90	41.55	42.20			41.35	40.55	40.35		D		
	Weight 388	350	326	312	295	280	273	262			210	197	167				
N 3 {	Temperature 42.20	42.55	41.65	42.20	42.75	42.65	42.20	42.10			41.45	41.10	40.20	42.20			
	Weight 364	330	306	292	272	270	258	250			226	212	195	208**	R		
B 15 {	Temperature 43.00	39.75	39.10	39.65	39.70	40.00	40.75	40.45	40.20	40.33	41.05	42.20					
	Weight 370		P 314	P 308	P 284	P 272	L 257	L 245	S 235	S 265	S*	R					
B 16 {	Temperature 42.45	40.45	40.00	39.33	39.70	39.75	39.45	39.10	39.65	40.00	40.45			39.90	40.00		
	Weight 400		P 352	P 344	P 326	P 312	P 300	P 283	P 265	P 255	P	P	P 237	P 222	P	D	
B 18 {	Temperature 42.45	39.65	40.65	39.75	39.55	39.55	39.55	39.55	39.55								
	Weight 350		P 296	P 290	P 264	P 254	P 240	P 225	P 200	D							
B 7 {	Temperature 42.20	39	P	P	39.65	39.90	40.00	39.45	38.90	39.45	39.00	40.10	38.90	39.75	39.65	39.45	
	Weight 300		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P 186
																	P 168 D

D, dead; L, lying; P, prostrate; R, recovered; S, standing.

Records taken every 24 hours between 4:30 and 5:30 p.m.

No food or water given to any bird except as noted.

N 1, N 2 and N 3—controls.

B 15 received 150 mgm. barbital per kilo.

B 16 received 300 mgm. barbital per kilo.

B 18 received 343 mgm. barbital per kilo.

B 7 received 225 mgm. barbital per kilo.

*Drank water during preceding twenty-four hours.

**Drank water and took food during preceding twenty-four hours.

Weight in grams, body temperature in degrees Centigrade.

series 7 adult pigeons were used. Four of these received standard doses of barbitol by intra-abdominal injection and the other 3 were carried as controls. None of the 7 birds received either food or water from the beginning of the experiment until the close of the test (see chart 2 and table 3). The undrugged birds remained active for twelve days, and were quite restless after the sixth day. During the twelfth day they became quiet, stood with eyes closed and feathers ruffled, and showed marked muscular weakness. One of the 3 died on the thirteenth day. The other 2 were given water at once, but a second bird died on the fourteenth day in spite of the fact that it had taken a few cubic centimeters of water about noon on the thirteenth day. The third bird ate a small quantity of grain and drank several cubic centimeters of water late in the afternoon of the thirteenth day, and that bird made a complete recovery. Of the 4 barbitolized birds 1 died on the tenth day, 1 on the sixteenth day and 1 on the seventeenth day. The fourth recovered on the twelfth day.

The body weights of all 7 pigeons in this experiment showed the same type of daily decline throughout the test, but the barbitolized birds lost weight at a slightly slower rate than the controls. Several factors contributed to this difference in favor of the drugged birds. The controls were active and restless, standing and moving much of the time during the first twelve days. The barbitolized birds on the contrary were prostrate after the third hour of the experiment and remained so until they died, with the exception of B 15. This bird recovered on the twelfth day and was able to stand after the eighth day, although it was not active until the eleventh day. Oxygen consumption tests made on birds in barbitol narcosis (data discussed elsewhere) gave an oxygen consumption rate some 20 per cent lower than the normals of the same birds before injection of barbitol, indicating that in addition to being less active than the control birds the barbitolized birds were maintaining a lower metabolism level. There was also a noticeable difference in the amounts of material excreted by the birds of the two sets. The control birds after the first two or three days voided relatively little material, and particularly little fluid, while the barbitolized birds discharged

not only much larger quantities of excreta, but excreta of high water content throughout the experiment. This was true of all barbitalized birds observed, and suggests a possible diuretic action of barbital for birds. Hoffman (4) has stated that small doses of barbital are somewhat diuretic.

The changes in body temperature of the birds of the two series followed quite different lines. The average body temperature of the pigeons to which barbital was given fell from 42.53° to 39.57°C . in the first forty-eight hours, and the 3 birds of this set which died maintained a temperature between 39.10° and 40.65°C . until death. Pigeon B 15 gradually returned to normal body temperature as it recovered, reaching the normal, 42.20°C ., on the twelfth day. The average body temperature of the control birds fell gradually from 42.68° to 41.45°C . in the first eleven days, after which there was a more rapid decline. The temperature of the control birds did not drop below 40.50°C . until the thirteenth day, i.e., about twelve hours before 2 of them died. The temperature of N 3, the control which recovered, was 40.20°C . on the thirteenth day, and after taking food and water this pigeon returned to a normal temperature of 42.20°C . during the next twenty-four hours.

It is evident from this experiment that the low body temperatures maintained by birds under the influence of barbital were not the result of starvation and water deprivation alone, during at least the first ten to twelve days. The starving control birds maintained a body temperature two or three degrees higher than the barbitalized birds for ten to eleven days, i.e., until within about forty-eight hours of death. Even the last temperature readings of the two control birds, recorded about thirty minutes before they died, were higher than the average temperatures maintained by the barbitalized birds during narcosis.

The combined action of starvation, water deprivation and barbital however may have caused the death of birds remaining in barbital narcosis for ten days or more, as of the 6 birds (from all experiments) which were in barbital narcosis for ten days or longer, 3 recovered on the tenth, eleventh and twelfth days respectively, and 3 died on the tenth, sixteenth and seventeenth

days. If the tenth day of starvation and water deprivation be considered as grossly marking the beginning of collapse from starvation, recovery from a dose of barbitol which would render the pigeon incapable of taking food and water for ten days, would hardly be expected.

RESPIRATORY RESPONSES

During the first hour after the injection of barbitol the respiration rate of about one-half of the pigeons observed rose from a normal of 20 to 22 respirations per minute to 40 per minute or higher. The respiration rate of the other birds made very little change during the first thirty to sixty minutes. In pigeons remaining in barbitol narcosis for eight hours or more the respiration rate fell slightly, as the respiration depth was increased in most birds after the first stages of barbitol action were passed. The respiration rate continued to fall slowly to 12 to 16 per minute after several days in birds which had received fatal doses, and there was usually an appreciable rise in respiration in pigeons recovering about the time they were able to stand again.

DOSAGE

In table 4 the various doses of barbitol used in these experiments are listed. From these tests the minimum fatal dose of barbitol when given by intra-abdominal injection was considered to be between 225 and 250 mgm. per kilo of body weight for the adult pigeon. Some deaths resulted from 225 mgm. per kilo, but a larger number of pigeons recovered from doses of that size although the narcosis produced lasted in every case for three days or longer. Four squabs, 2 three weeks old (S 1 and S 2) and 2 two weeks old (S 3 and S 4), were tested with 225 mgm. per kilo doses. All 4 squabs recovered from the first doses in three days, and 3 of the 4 recovered from their second doses in a similar period. The fourth squab 'S 2, died from its second dose of 225 mgm. per kilo on the fourth day.

Compared with the published dosages for other animals (table 5) the minimum fatal dose for the pigeon is smaller than that for the

TABLE 4

PIGEON NUMBER	NUMBER OF DOSE	BARBITAL	INJECTED INTO	DAY OF RE- COVERY	DEAD
Adult pigeons					
		<i>mgm. per kilo</i>			
B 14	1st	100	Abdomen	4th	
B 2	1st	102	Crop	2d	
B 1	2d	102	Crop	2d	
B 5	1st	105	Abdomen	4th	
B 15	1st	150	Abdomen	12th	
B 2	4th	200	Abdomen	2d	
B 10	1st	200	Abdomen	5th	
B 10	2d	200	Abdomen	4th	
B 6	1st	209	Abdomen	4th	
B 2	2d	217	Crop	3d	
B 5	3d	225	Abdomen	5th	
B 8	2d	225	Abdomen	5th	
B 5	2d	225	Abdomen	8th	
B 8	1st	225	Abdomen	9th	
B 12	1st	225	Abdomen	9th	
B 6	2d	225	Abdomen	11th	
B 7	1st	225	Abdomen		17th day
B 2	5th	225	Abdomen		8th day
B 11	1st	225	Abdomen		3d day
B 2	3d	226	Abdomen	5th	
B 1	1st	242	Crop	10th	
B 1	3rd	268	Abdomen		2 hours
B 3	1st	273	Abdomen		3 hours
B 17	1st	275	Abdomen		3rd day
B 16	1st	300	Abdomen		16th day
B 18	1st	343	Abdomen		10th day
B 19	1st	400	Abdomen		30 minutes
B 12	2d	400	Abdomen		80 minutes
B 20	1st	500	Abdomen		17 minutes
B 6	3d	500	Abdomen		55 minutes
B 21	1st	600	Abdomen		10 minutes
Squabs					
S 1	1st	225	Abdomen	3d	
S 2	1st	225	Abdomen	3d	
S 3	1st	225	Abdomen	3d	
S 4	1st	225	Abdomen	3d	
S 1	2d	225	Abdomen	3d	
S 2	2d	225	Abdomen		4th day
S 3	2d	225	Abdomen	3d	
S 4	2d	225	Abdomen	3d	

TABLE 5

ANIMAL	MINIMUM FATAL DOSE	INJECTION	AUTHORITY
	<i>mgm. per kilo</i>		
Dog.....	450		Bachem (1)
Cat.....	300-350	Subcutaneous	Roemer (7)
Rabbit.....	400	Subcutaneous	Roemer (7)
Rabbit.....	400-450		Bachem (1)
Pigeon.....	225-250	Intra-abdominal	Present experiments
Frog.....	1500	Dorsal lymph sac	Roemer (7)

dog, cat, rabbit or frog. The pigeon and mammals are conspicuously more sensitive to this drug than the frog. As the action of barbitol involves such profound changes in body temperature of the isothermous animals it is interesting to note that the pigeon which has a normal body temperature of about 42.2°C. is more sensitive to barbitol than the mammals which have normal temperatures several degrees lower.

SUMMARY

1. Intra-abdominal injections of barbitol produced in pigeons a definite series of changes in position and muscular activity, terminating in a fairly uniform and usually much prolonged narcosis.

2. This narcosis lasted from two to twelve days in birds recovering, and from one to seventeen days in those receiving fatal doses. In general the duration of the narcosis varied with the amount of barbitol given below 300 mgm. per kilo of body weight.

3. These injections of barbitol also produced a rapid and pronounced lowering of body temperature of pigeons, which averaged 0.8° during the first fifteen minutes, 1.7° during the first thirty minutes and 2.8°C. during the first hour.

4. The minimum body temperature in the first stage was recorded about two and one-half hours after the injection of barbitol and the depression averaged 3.8°C. at that time.

5. The minimum body temperature during the entire period of barbital narcosis appeared in the first eight hours in over half the cases observed, but was delayed in some for forty-eight hours or more.

6. Compared with control pigeons which were given neither food nor water for twelve days pigeons in barbital narcosis maintained a distinctly lower body temperature and lost weight at a slightly slower rate.

7. The minimum fatal dose of barbital given intra-abdominally was found to be between 225 and 250 mgm. per kilo of body weight for adult pigeons.

8. Pigeons were more sensitive to barbital than cats, dogs, rabbits or frogs, although the depressions of body temperature produced in pigeons were not as great as those reported for some mammals.

9. Satisfactory surgical anesthesia in pigeons was not obtained by the use of barbital alone.

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THE RESPIRATORY EXCHANGE AND BLOOD SUGAR CURVES OF NORMAL AND DIABETIC SUBJECTS AFTER EPINEPHRIN AND INSULIN

RICHARD S. LYMAN, ELIZABETH NICHOLLS AND WM. S. McCANN

From the Chemical Division, Medical Clinic, the Johns Hopkins Hospital, Baltimore, Maryland

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The physiological effects of insulin have been studied intensively since its production by Banting, Best, Macleod, Collip and their collaborators (1). They have found that blood sugar is lowered, the respiratory quotient raised and that glycogen is deposited in the liver after suitable injections of this substance. They have also investigated the antagonistic effect of epinephrin and insulin, especially in regard to the behavior of blood sugar. In this connection a series of experiments was conducted in this clinic in which the effect of insulin and epinephrin, alone and in combination, was observed on 6 normal men and 8 diabetic patients. These experiments form the basis of this report.

LITERATURE

The literature on insulin, in so far as it bears on this paper, has been referred to above. A review of the status of insulin, with complete bibliography up to April, 1923, is given in the Journal of the American Medical Association (2).

The response of normal individuals to epinephrin has been widely investigated from many sides. Brief reviews of the literature of its effects on respiratory exchange are given by Tompkins, Sturgis and Wearn (3) and by Sandiford (4). In these articles as well as in an article by Boothby and Sandiford (5) and one by Abelin (6), experiments are reported which invariably show an increase in heat production and with but few exceptions a rise of R.Q. following epinephrin. Börnstein and Müller (7)

reported carefully controlled experiments from which they concluded that, in view of a decrease of alveolar tension and an increase of blood sugar at the time when the highest R.Q.'s appeared after epinephrin, those R.Q.'s are brought about by overventilation. Hartman (8) reviewed the influence of epinephrin on sugar metabolism from the work of Blum in 1902 to the present time. He discusses the hyperglycemia and glycosuria which are obtained by injections of suprarenal extract and also calls attention to the work which showed the effect of epinephrin on blood sugar to be greater when larger amounts of glycogen are stored. He concludes with the statement that although suprarenal extract does not appear to be essential to the production of the hyperglycemias of asphyxia, ether and piqûre and perhaps other causes, it does stimulate glycogenolysis and may assist in the glycogenolysis produced by these other means.

Experiments with epinephrin in diabetic patients have been reported from time to time. Veil and Reisert (9) refer to the previous literature and discuss their own observations on this subject. They describe different types of reaction to epinephrin in diabetic patients. They find that mild cases of diabetes gave a hyperglycemia similar to that of normal subjects, that 5 patients with severe diabetes gave a more marked hyperglycemia, and finally that there was a group in which the blood sugar fell at first and then rose above the initial level. All the patients in that last group had some complication in the nervous system. Brösamlen (10) also reports the effect of epinephrin on the blood sugar in diabetic patients. He observed different reactions in different individuals: in some instances there was only a slight increase of blood sugar, in others a drop followed by a rise, and again in other patients there was an extreme elevation of blood sugar. He states that these hypoglycemic curves bore no relation to the severity of the diabetes.

Prior to the investigations of the Toronto school, which are referred to above, the most convincing work on the antagonism of extracts made from the adrenal glands and from the pancreas is that of Achard, Ribot and Binet (11) who brought on a marked hyperglycemia in dogs by giving a glucose-epinephrin mixture

and then reduced the blood sugar by adding a fresh pancreas extract to it. Ross and Davis (12) recently performed experiments on dogs from which they conclude that adrenalin and the pancreatic hormone contained in fresh pancreas preparations react together in an antagonistic balance and the one in excess affects the glycogen stores of the body.

METHODS

The subjects used in these experiments were taken in the morning in postabsorptive state and in complete muscular relaxation. Further details of the technic of preparation for these investigations are given by McCann and Hannon (13). Preliminary determinations were made on the respiratory exchange, blood-sugar, urinary nitrogen and in most cases on the pulse rate and blood pressure. Then epinephrin was injected subcutaneously and the respiratory exchange again studied at varying intervals beginning eight to ten minutes after the injection and lasting about an hour. The preparation was Armour's 1:1000 solution of Suprarenalin, the usual dose being 0.5 cc. The same subjects were observed under similar conditions on subsequent days when insulin was injected intravenously, and finally when both insulin intravenously and epinephrin subcutaneously were administered on the same morning. The respiratory exchange was followed in these experiments at intervals for two to three hours after the injections. The insulin used was the product obtained from Eli Lilly and Company as "Iletin," the dosage varying from $2\frac{1}{2}$ to 5 units with normal subjects and from 10 to 15 units with diabetic patients. The method used for determining the respiratory exchange was the open-circuit Tissot method, the gas being analyzed in a modified Henderson-Haldane apparatus and the calculations of this indirect calorimetry made by the method of Zuntz and Schumburg, as described by McCann and Hannon (13). Alveolar air determinations, when made, were carried out by a modified Haldane method. At frequent intervals during the experiment samples of blood were taken and blood sugar determinations were made according to the method of Folin and Wu (14). Coincident pulse and blood pressure charts

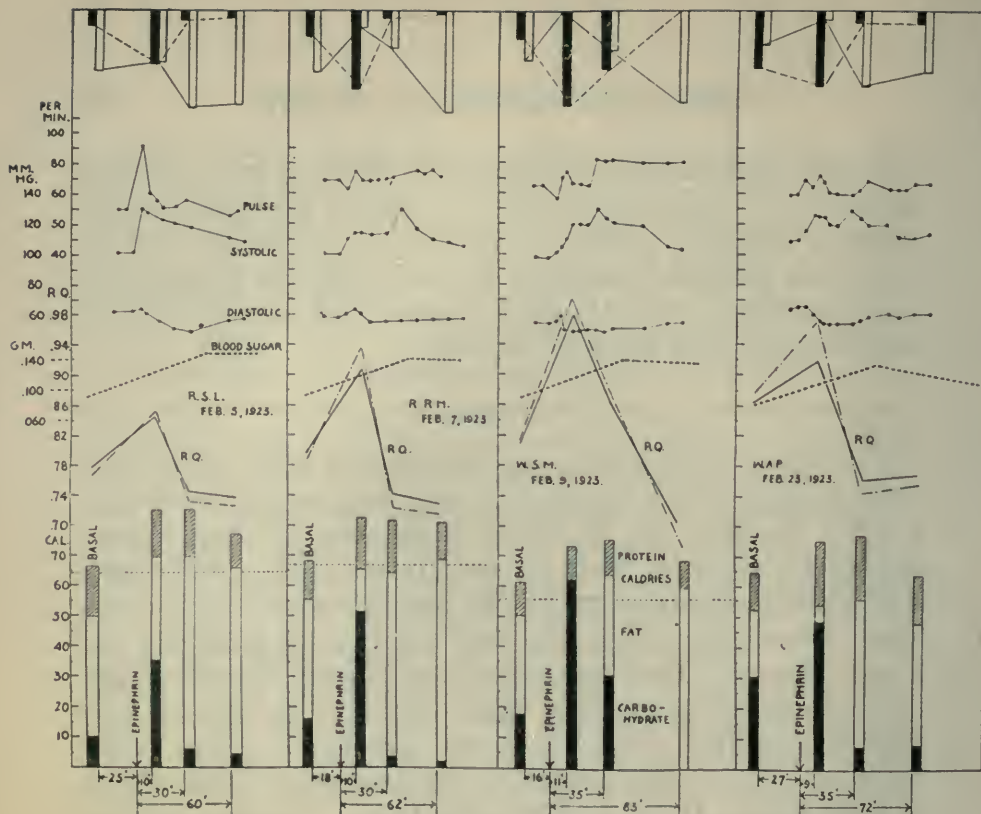


CHART 1. 0.5 CC. EPINEPHRIN IN NORMAL SUBJECTS

EXPLANATION OF GRAPHIC RECORDS

The data shown in these graphs is plotted to the same scale throughout.

The abscissa units represent time in minutes, labeled arrows indicating the injections of epinephrin or insulin.

Items on the ordinate axis from the bottom up are represented as follows:

1. Calories per hour in each successive respiratory period as vertical bars.

A horizontal broken line gives the normal standard of calories per hour for the sex and age and surface area of the subject in each experiment, according to the method of Aub and DuBois.

When the bars are subdivided, black represents calories derived from fat and white calories from carbohydrate metabolism, as calculated from the non-protein R.Q. The cross-hatched portion denotes calories derived from protein as calculated from the urinary nitrogen.

2. Total R.Q. at each respiratory period as solid lines. Broken lines accompanying the graph of total R.Q. represent the non-protein R.Q., when plotted.

3. Broken line as blood sugar in grams of sugar per 100 cc. of blood.

4. Diastolic and systolic blood pressure as solid lines on which the time of successive readings is indicated by dots.

5. Pulse rate as solid lines with dots indicating the time of consecutive determinations.

6. In some of the graphical records, the comparison of fat (black) and carbohydrate (white) metabolism during each respiratory period as vertical bars at the top of the diagram. This data is identical with that given in the vertical bars at the bottom of the graph and is drawn to the same scale.

were kept in most instances, and the subjective symptoms of the patient were recorded. In order to save space and to clarify the presentation of results the experimental data have been recorded entirely by means of graphic charts.

EPINEPHRIN IN NORMAL SUBJECTS

In the studies described in this paper, 0.5 cc. of epinephrin was administered subcutaneously to 6 normal men—4 physicians and 2 medical students. All 6 subjects gave a similar reaction. This can be seen in chart 1, in which the data of the first four

TABLE 1

Data obtained from W. A. P. in an experiment performed on February 23, 1923, as recorded in chart 1

PERIOD	CAL- ORIES PER HOUR	R. Q.	MINUTE VOL- UME	TIDAL AIR	DEAD SPACE	ALVE- OLAR VENTI- LATION PER MINUTE	ALVE- OLAR CO ₂	ALVE- OLAR CO ₂ TEN- SION	REMARKS
			cc.	cc.	cc.	cc.	per cent	mm. Hg	
I	65.5	0.862	7126	421	210	3588	6.39	47.1	Basal
II	76.3	0.920	8771	495	244	4431	6.20	46.3	0.5 cc. epinehrin
III	78.5	0.763	8376	429	223	3986	6.09	45.4	9 to 15 minutes after
IV	65.1	0.770	7667	418	235	3183	64.3	48.0	35 to 41 minutes after
									72 to 78 minutes after

experiments with epinephrin on normal men is graphically presented. There was a prompt rise of the total R.Q., which was most marked about ten minutes after the epinephrin, the extent of this rise depending upon the individual. A distinct increase of heat production followed the injection of epinephrin, reaching its maximum about thirty minutes after the injection and remaining above the basal level for over an hour. As calculated from the non-protein R.Q., there appeared to be an immediate and marked rise in carbohydrate metabolism with its maximum about ten minutes after the epinephrin. In an effort to determine to what extent the elevation of quotients was due to overventilation, an experiment was performed on a normal man, W. A. P., in which the alveolar CO₂ tension, effective alveolar ventilation and dead space were calculated. The results of these experiments are shown in table 1. The decrease

in alveolar CO_2 tension shown in this experiment indicates that overventilation had occurred. Epinephrin may have increased the irritability of the respiratory center in this case. The higher respiratory quotients after adrenalin may be wholly or partly due to this effect. There is a rough parallelism shown between effective alveolar ventilation and oxygen consumption. The evidence given in this experiment makes it quite doubtful whether the higher respiratory quotients after adrenalin indicate a relative increase in carbohydrate oxidation. This view corresponds with that of Börnstein and Müller. If it is correct, the calculation of the proportions of foodstuffs oxidized after the injection of adrenalin may lead to erroneous conclusions.

An elevation of blood sugar followed the injection of 0.5 cc. epinephrin in all normal subjects. Since Brösamlen (10), Bjure and Svensson (15) and others have reported that the maximum hyperglycemia occurs from forty-five to sixty minutes after the administration of the drug, blood was taken in this series between forty and fifty minutes and again from sixty to one hundred and ten minutes after epinephrin. The average maximum rise of blood sugar at those intervals for the 4 controls presented in chart was 58 per cent. Two other normal subjects at a later date showed percentage increases of 42 and 237 per cent respectively. The sugar remained above normal limits in all cases of this series for over one and one-half hours.

The changes of pulse rate and blood pressure and the subjective symptoms varied in different individuals and the degrees of response to epinephrin in these aspects as well as in the respiratory exchange and blood sugar did not always run parallel to each other in the same individual.

EPINEPHRIN IN DIABETIC PATIENTS

The reaction to epinephrin of the diabetic patients differed in some respects from that of the control cases. The R.Q. did not rise as high as in the normal subjects. There was only one diabetic patient whose R.Q. went up as many points as did that of the minimum control. As regards the total heat production the 8 diabetic patients formed two groups: 4 showed an increase from

6 to 18 per cent following administration of epinephrin, the rest ranged from 29 to 43 per cent above their basal metabolism. The equivalent rise which occurred in 5 normal cases lay between 17 and 33 per cent above the basal heat production. In the diabetic patients of this series the hyperglycemia due to epinephrin did

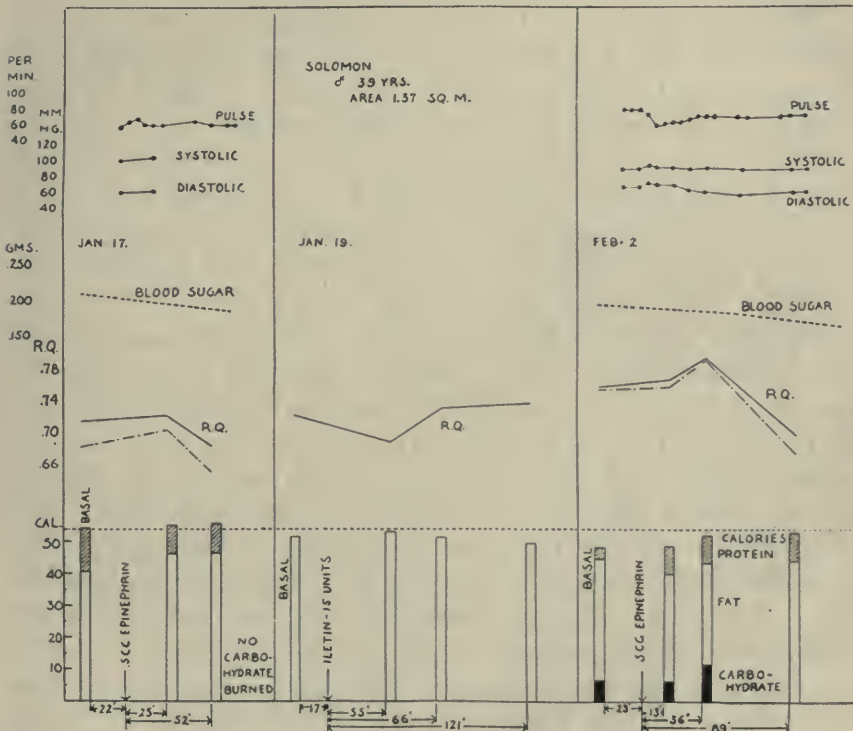


CHART 2. 0.5 CC. EPINEPHRIN ON A DIABETIC PATIENT, SHOWING DECREASE OF BLOOD SUGAR

Slight fall of R.Q. in first respiratory period after 15 units of insulin intravenously.

not appear so soon or rise so high as in the controls, there being one exception. For example, the highest blood sugar level of 4 diabetic patients was reached one hundred to one hundred and ten minutes after the administration of the drug. In contrast to this the peak in normal cases came forty to fifty minutes after the epinephrin, and in the case of one diabetic patient who

showed no circulatory or subjective response to epinephrin, the blood sugar fell 0.028 per cent in two hours. The experiment was repeated on this patient with identical results, as is shown in chart 2.

INSULIN IN NORMAL SUBJECTS

The reaction of normal subjects to insulin was observed in 5 experiments, only 3 of these, however, having the complete data as outlined in the introduction to this paper. Usually $3\frac{1}{2}$ units were given intravenously. This dosage brought out a moderate reaction. One subject received $2\frac{1}{2}$ units with very little reaction, while 5 units, given to another subject, required the subsequent administration of sugar to relieve the symptoms of hypoglycemia.

In all the experiments of this group there was a marked rise of R.Q. The smallest increase was from 0.847 to 0.877, while the largest was from 0.794 to 0.945. The maximum in each case appeared about thirty minutes after the intravenous injection of $3\frac{1}{2}$ units of insulin. In the case of one control, who had proven to be very sensitive to epinephrin, the R.Q. dropped slightly after a ten minute interval on one occasion and after a twenty minute interval in a repetition of the experiment. His R.Q. then rose as in the other experiments of this group. An increase of heat production occurred in all cases, ranging between 2.5 and 17.7 per cent, and averaging 10.6 per cent, above the basal level. The maximum increase occurred from ten to sixty minutes after the administration of insulin, but one and one-half to two and one-half hours later the heat production lay at or below the basal figure.

Following intravenous injection of insulin there was a rapid fall of blood sugar which was at its lowest level from twenty to thirty minutes after injection. The extent of the drop varied with the individual. The lowest figure obtained after $3\frac{1}{2}$ units was 0.025 per cent. The experiment was repeated one week later on the same subject with the same dosage and the lowest blood sugar was read as 0.031 per cent. In both cases duplicate readings were made against two standards with good checks and the highest figure was reported. The minimal fall in blood sugar

level which occurred in another normal subject who received $3\frac{1}{2}$ units, was from 0.079 to 0.047 per cent. The most striking features brought out by these experiments with normal subjects were the rapidity of the decrease of blood sugar percentage and the abruptness of the return to normal level. By forty minutes after the intravenous injection of $3\frac{1}{2}$ units of insulin, the blood sugar was already rising in every case and by $2\frac{1}{2}$ hours it had returned to about the normal figure.

The systolic blood pressure in normal men after the injection of insulin usually rose from 2 to 16 mm. Hg. The diastolic pressure usually fell. As a rule the pulse rate increased 2 to 16 beats per minute, reaching its maximum about thirty-five to forty minutes after the injection. This increase was never as marked as when epinephrin alone was given to the same subject. The rate of respiration was in some instances somewhat higher (6 to 10 per minute) and in others slightly lower (1 to 4 per minute) following $3\frac{1}{2}$ units of insulin.

In the case of W. A. P., who volunteered to take 5 units of insulin, there was a marked reaction which suddenly made its appearance twenty minutes after the intravenous injection. He presented a typical picture of hypoglycemia, as described by Banting, Campbell and Fletcher (16) with weakness, clouding of consciousness, pallor and profuse perspiration. His blood pressure rose abruptly from 106/65 to 140/56, his respiratory rate increased from 15 to 23 per minute, and by 28 minutes after the injection his blood sugar was found to be 0.027 per cent. He was then given sugar by mouth and the symptoms wore off in about two hours.

Marked subjective symptoms were twice noted to occur on the initial injection of insulin, while on repetition of similar experiments the subjective sensations were minimal or absent, although the blood sugar fell practically as low as on the initial injection. The subjects in these experiments noticed the contrast between the well known symptoms following the administration of epinephrin and the train of symptoms which appeared after insulin: weakness, chills or sweating, dimming of sense percep-

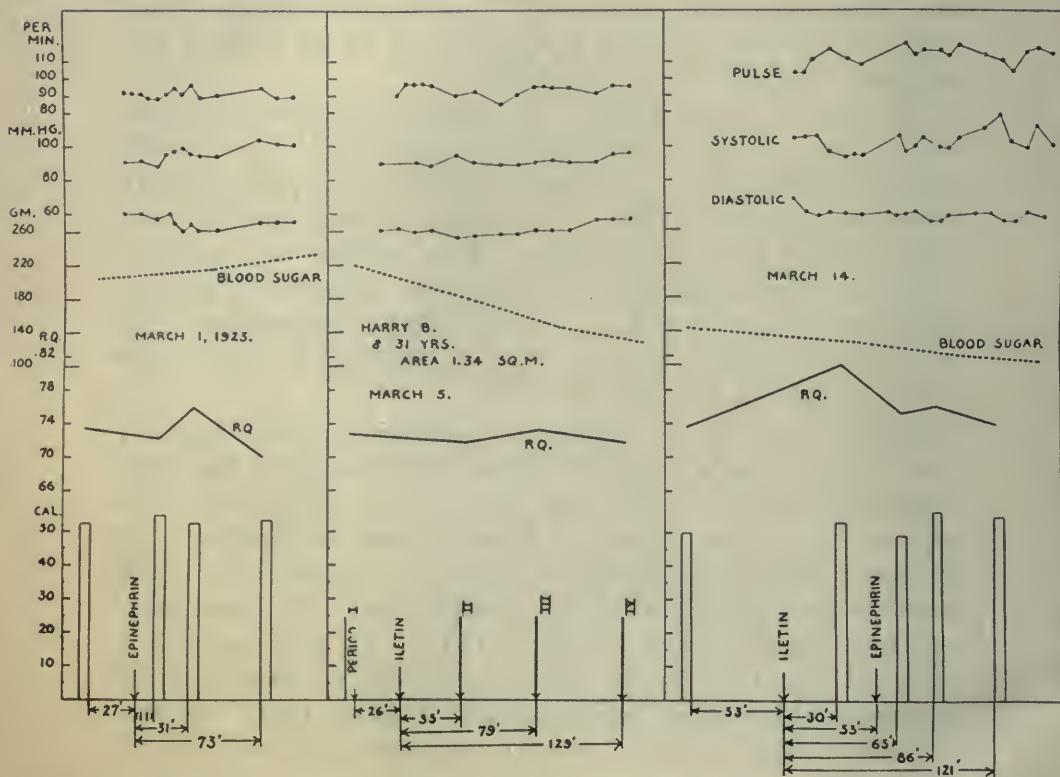


CHART 3. EPINEPHRIN AND INSULIN IN A SEVERE DIABETIC SUBJECT, COMPLICATED WITH PULMONARY TUBERCULOSIS

tions, mental haziness and wandering attention—all of which were due, presumably, to the hypoglycemia.

INSULIN IN DIABETIC PATIENTS

Seven diabetic patients who had been given epinephrin received insulin at a later date. The usual dose was 10 units intravenously. The effect was similar to that described in the publications of the Toronto investigators (2), but there is the difference that in their clinical studies insulin was injected subcutaneously in most cases, as is done in the wards in this Clinic. In this series of experiments the reaction of diabetic subjects was similar to that of the controls but the maximum effects were slower in appearance and the decrease in blood sugar lasted for a much longer time. The R.Q. invariably rose sooner or later after insulin. The maximum rise occurred later than with the normal men and was somewhat less than in the control cases. The smallest increase was from 0.783 to 0.787, while the largest was from 0.749 to 0.841. The extent of increase of heat production produced by insulin ranged from 2.9 to 19.6 per cent, with an average of 11.6 per cent, above the basal level. This average increase is 1 per cent above the corresponding figure obtained with the normal subjects. Within two hours after receiving the insulin, however, the heat production had fallen to or below the basal determination in all cases.

With 3 diabetic subjects the R.Q. decreased slightly during the first half hour after insulin and then increased. This occurred on the initial injection but after a course of insulin therapy this phenomenon disappeared. Two of these subjects, presented in charts 2 and 3, showed minimal reactions to both insulin and epinephrin, while the other gave a relatively slight response to insulin but a marked one to epinephrin.

The blood sugar percentage always decreased but there was considerable individual variation in the extent of the decrease. In the case of 4 diabetic patients whose morning fasting blood sugar was in every case above 0.200 per cent, 10 units of insulin intravenously caused a drop of 0.095 to 0.173 per cent, with an average decrease of 0.122 per cent. It is interesting to note that the patient who showed the greatest increase of R.Q. and

H.A.
Male, Age 17.
Diabetic.

BLOOD-SUGAR AFTER INSULIN.
(10 Units intravenously)

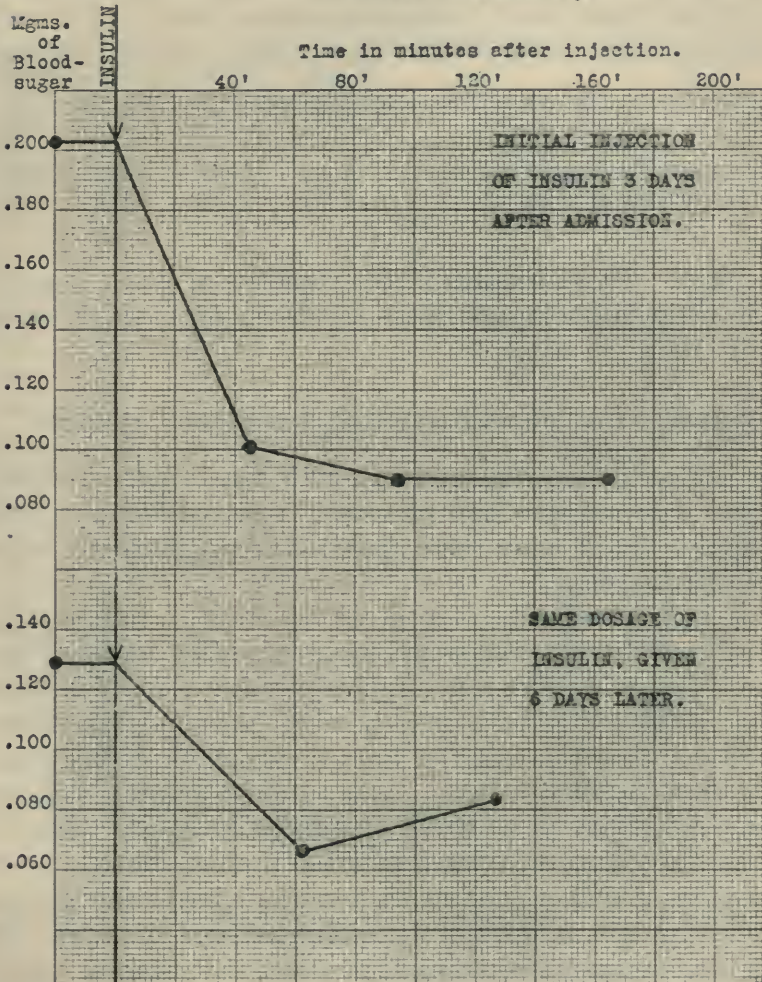


CHART 5. COMPARISON OF THE EFFECT OF 10 UNITS OF INSULIN ON BLOOD SUGAR IN A DIABETIC PATIENT THREE DAYS AFTER ADMISSION AND AFTER A PERIOD OF CLINICAL IMPROVEMENT

as clinical improvement occurred, and this condition of hypoglycemia was terminated much more quickly by the increase of the sugar percentage than occurred before such improvement

TABLE 2

	AVERAGE MORNING FASTING BLOOD- SUGAR	AVERAGE LOWEST LEVEL AFTER INSULIN	AVERAGE EXTENT OF DROP IN GRAMS OF SUGAR	AVERAGE PER CENT- AGE DROP
4 diabetic patients (dosage 10 units) . . .	0.216	0.094	0.122	55.8
5 controls (dosage 3½ units)	0.082	0.036	0.046	56.1

took place. Those changes in the response of blood sugar to insulin with clinical improvement are shown, for example, in the case of H. A. in chart 5.

COMBINED INSULIN AND EPINEPHRIN IN NORMAL SUBJECTS

It then seemed in order to give both insulin and epinephrin to those subjects who had previously received the two drugs separately. In all cases there appeared to be a distinctly antagonistic relationship.

Two normal subjects were given 3½ units of insulin intravenously and then, twenty-two to twenty-eight minutes later, 0.5 cc. epinephrin was injected subcutaneously. The epinephrin was administered at this time because previous experiments had shown that this was the time at which the blood sugar would reach its lowest level. One of these subjects had previously proven to be sensitive to epinephrin alone. When he received it twenty-two minutes after insulin, practically the same curve of R.Q. was obtained as when epinephrin was given alone, as appears in chart 6. In the other case there was relatively little response to epinephrin alone, and when it was injected twenty-eight minutes after insulin, there was actually a decrease of the R. Q., as is shown in chart 7. The calorogenic action of epinephrin following insulin was less than when it was given alone, with one exception to be described later. In each of the 2 experiments just mentioned, the curve of heat production

after the combined insulin and epinephrin injections lay between the lower one produced by insulin alone and the higher one by epinephrin, and it lay nearer to the one toward which the subject showed the greater tendency to react when receiving the two drugs separately. Chart 8 shows graphically the changes in

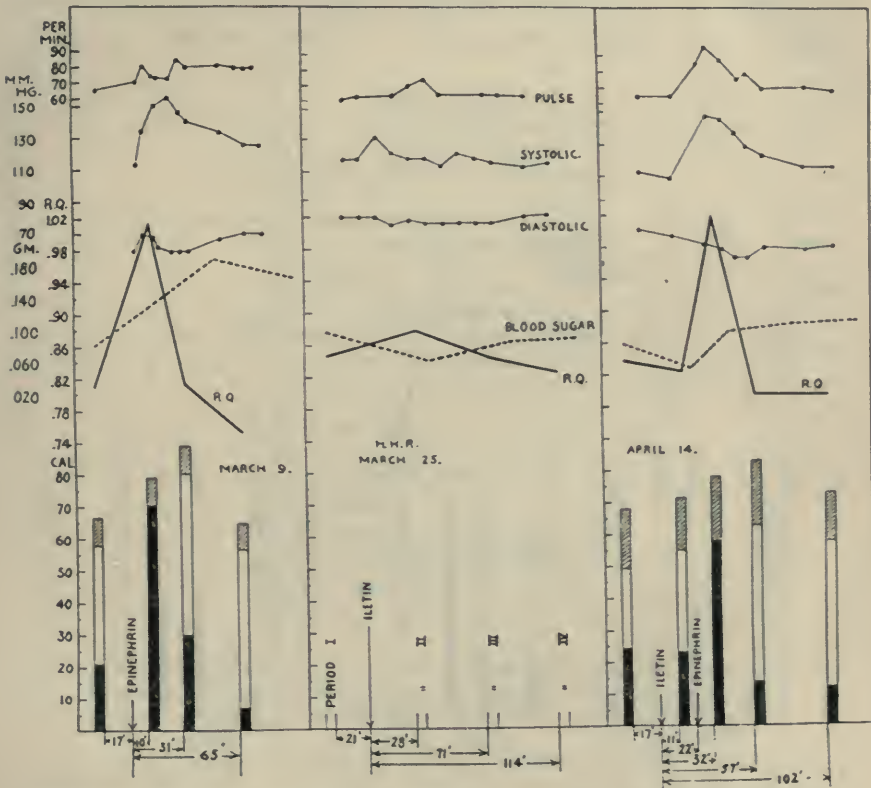


CHART 6. 0.5 CC. EPINEPHRIN AND 3½ UNITS OF INSULIN IN A NORMAL SUBJECT WHO IS SENSITIVE TO EPINEPHRIN

heat production of these two subjects in response to injections of insulin and epinephrin alone and in combination. The degree of hyperglycemia caused by epinephrin was not as great when preceded by insulin, the circulatory changes were not as marked, and the subjective symptoms were not as extreme as they were when the epinephrin was administered alone.

Two other normal subjects received simultaneous injections of $3\frac{1}{2}$ units of insulin intravenously and 0.5 cc. epinephrin subcutaneously. Both gave evidence of overventilation, most marked ten minutes after the injection. The respiratory quotients at that time were observed to be over 1.00 in each case.

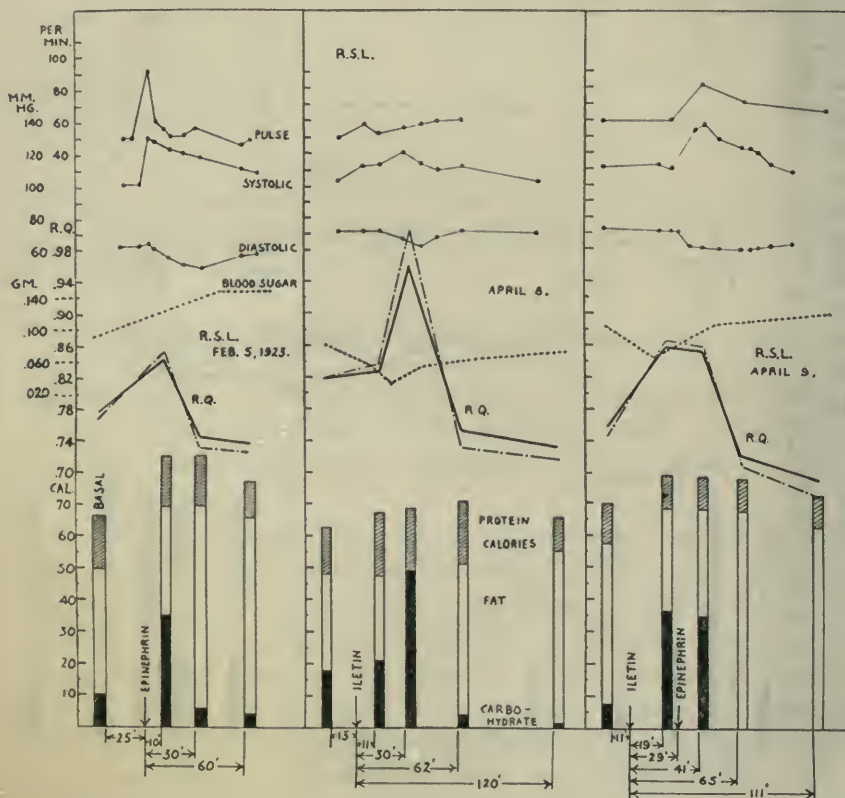


CHART 7. 0.5 CC. EPINEPHRIN AND $3\frac{1}{2}$ UNITS OF INSULIN IN A NORMAL SUBJECT WHO IS RELATIVELY INSENSITIVE TO EPINEPHRIN

Moreover, the maximum heat production rose to a higher percentage above the basal figures in both these cases than occurred in the experiments in which insulin preceded the epinephrin. When both drugs were injected simultaneously, the blood sugar of the subject, presented in chart 9, rose but did not reach the figure attained after epinephrin alone. His maximum heat

production occurred somewhat later than in either of his preliminary experiments and it proved to be 6.1 per cent higher than the maximum increase previously brought out by epinephrin given by itself. This was the only case in which insulin failed to restrict the calorigenic effect of epinephrin. After the combined dosage in that experiment, the respiratory rate rose from fifteen to 20 per minute. The other subject who received

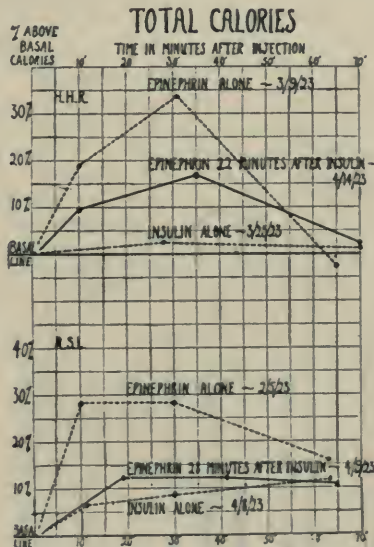


CHART 8. GRAPHICAL PICTURE OF THE INCREASED PERCENTAGE OF TOTAL CALORIES PRODUCED IN 2 NORMAL SUBJECTS BY EPINEPHRIN AND INSULIN, GIVEN ALONE AND IN COMBINATION

H. H. R. was relatively more sensitive to epinephrin and less so to insulin while R. S. L. responded relatively little to epinephrin and more to insulin when given separately. The increase of heat production after the combined injections was greater in the case of H. H. R. than of R. S. L., but in both cases it lay between that produced by epinephrin and that by insulin alone.

simultaneous injections had a slight drop of blood sugar after the injections, as is shown in chart 10. Although his maximum heat production rose, it remained 8.4 per cent below that which was calculated in the previous experiment with epinephrin alone. After the combined dosage his respiratory rate fell from 11 to 7

per minute. In the first of these 2 cases the epinephrin overbalanced the insulin, while in the other the reverse relationship held.

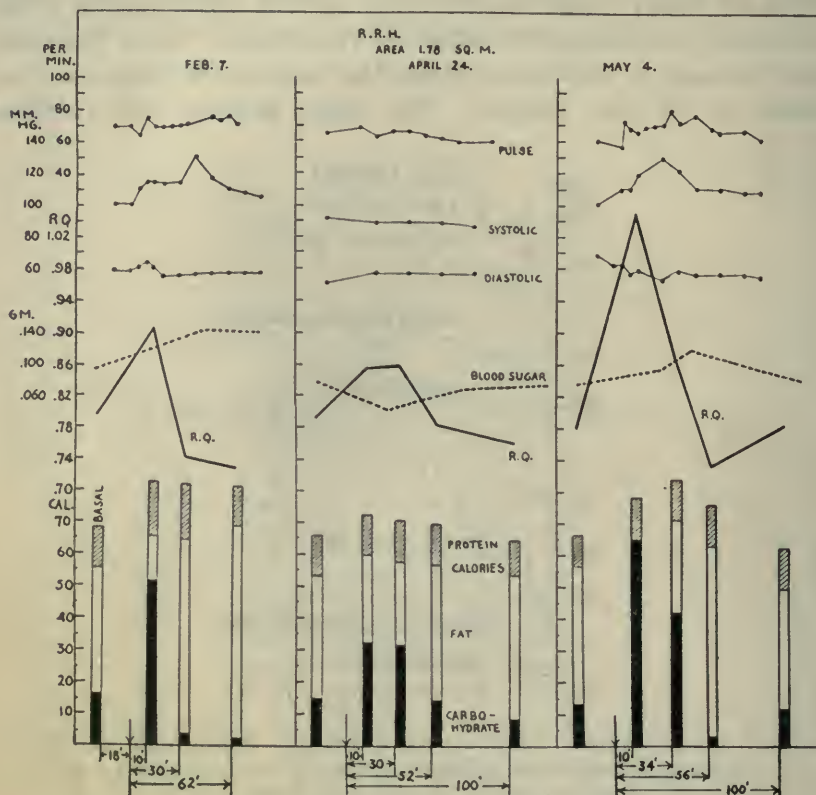


CHART 9. 0.5 CC. EPINEPHRIN AND $3\frac{1}{2}$ UNITS OF INSULIN IN A NORMAL SUBJECT

In the third experiment on May 4, epinephrin and insulin were given simultaneously.

COMBINED INSULIN AND EPINEPHRIN IN DIABETIC PATIENTS

When it came to similar experiments with diabetic patients, an interval of from thirty-nine to fifty-three minutes took place between the injections of 10 units of insulin intravenously and of epinephrin subcutaneously, in order to allow time enough for the insulin to lower the blood sugar to a considerable extent be-

fore adding the epinephrin. That is, of course, the relationship which is of the greatest immediate clinical importance, because epinephrin is not of any service to the practitioner until symptoms of hypoglycemia appear.

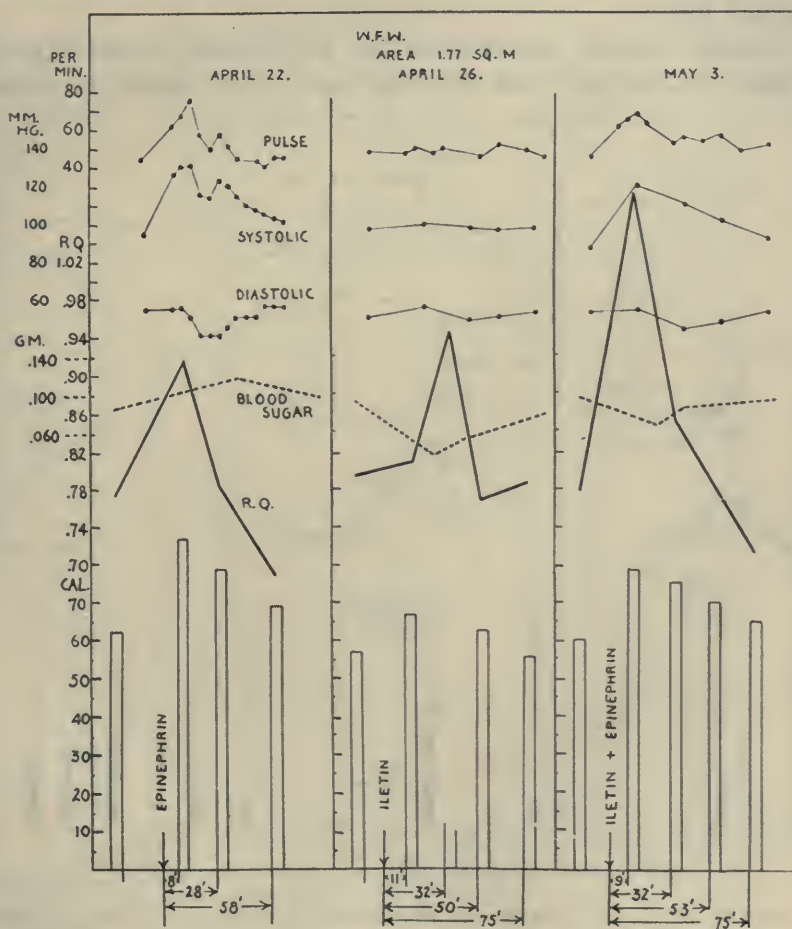


CHART 10. 0.5 CC. EPINEPHRIN AND $3\frac{1}{2}$ UNITS OF INSULIN IN A NORMAL SUBJECT

In the third experiment (on May 3), epinephrin and insulin were given simultaneously.

An antagonism between insulin and epinephrin appeared in the experiments with diabetic patients which was similar to that encountered with the normal individuals but was not always as marked. The effect of epinephrin following insulin in diabetic

patients seems to depend upon the individual sensitiveness to the drugs and also upon the condition of the patient as judged from the severity of the sickness, the presumable stores of glycogen, variations in the rate of absorption from the subcutaneous tissues, etc.

In this series of experiments the R.Q.'s were raised slightly during the first half hour after the injection of insulin, but when

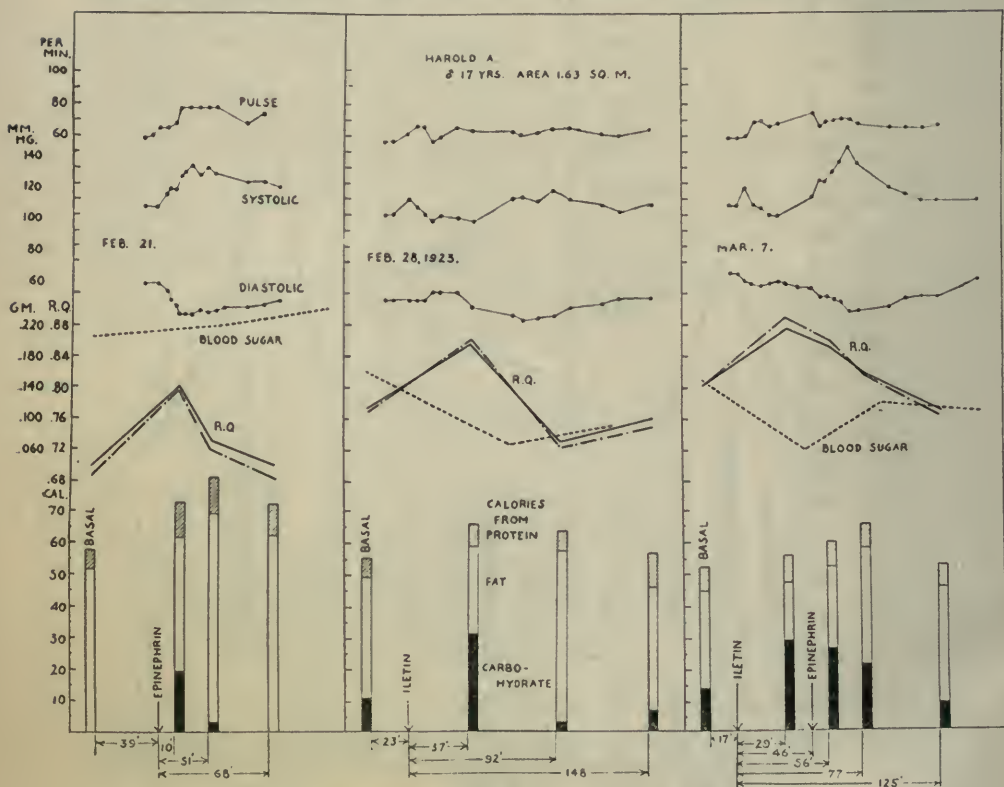


CHART 11. 0.5 CC. EPINEPHRIN AND 10 UNITS OF INSULIN IN A DIABETIC SUBJECT

epinephrin was administered later, they fell as often as not. The calorigenic effect of epinephrin proved without exception in this small group of diabetics to be less after insulin than when the former was given alone.

A very significant fact appeared in the behavior of the blood sugar. In 2 out of 4 cases, shown in charts 11 and 12, epinephrin after insulin brought the sugar curve up, but in the other 2 cases,

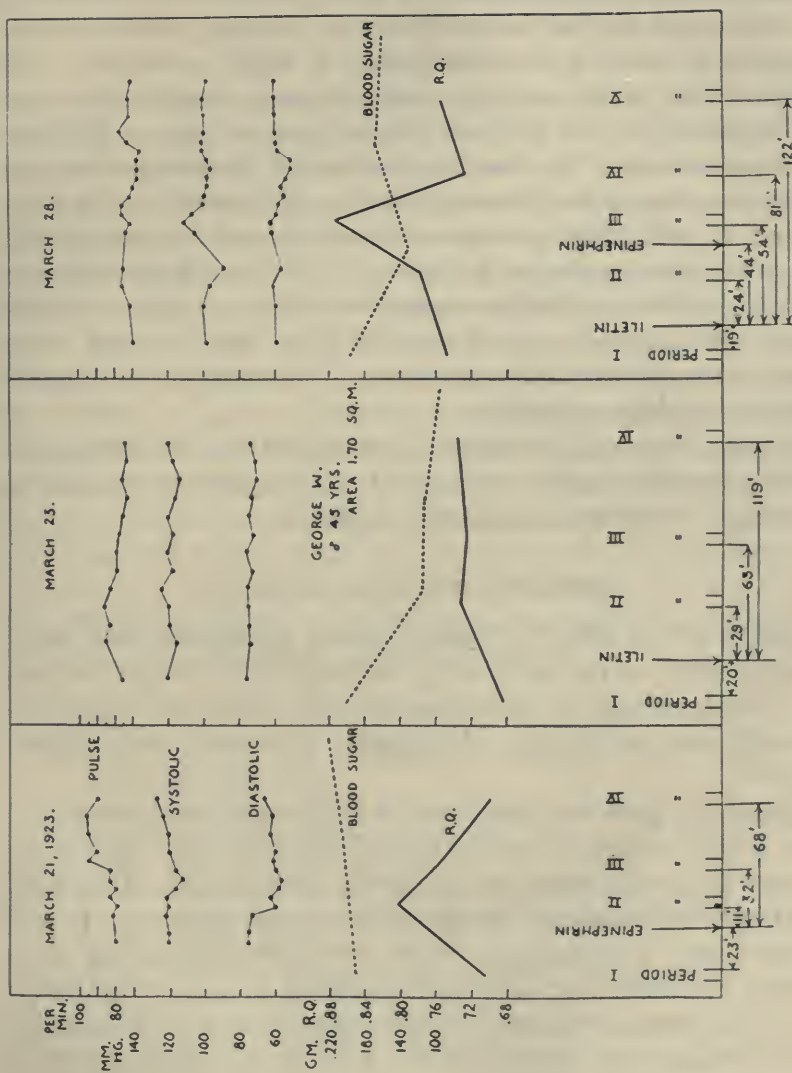


CHART 12. 0.5 CC. EPINEPHRIN AND 10 UNITS OF INSULIN IN A DIABETIC SUBJECT

the curve continued downward in spite of 0.5 cc. of epinephrin in one instance and 0.78 cc. in the other. One of those patients, appearing in chart 4, previously gave a slight reaction to epinephrin alone and a relatively marked one to insulin. He was not emaciated and he did not present a severe case of diabetes in other respects. The best explanation of the results obtained in this experiment is a constitutional sensitiveness toward insulin and an individual unresponsiveness toward the epinephrin. The other patient, shown in chart 3, had a much more severe diabetes, which was further complicated by pulmonary tuberculosis. His temperature of from 98.8° to 100°F. during these experiments changes the significance of the results as regards variations in heat production.

It seems, then, that epinephrin is not always to be relied upon to bring the blood sugar up in event of hypoglycemic symptoms brought on by insulin in diabetic patients.

SUMMARY AND CONCLUSIONS

Studies of the effect of subcutaneous epinephrin and intravenous insulin on the respiratory exchange, blood sugar, subjective symptoms and in most cases on the pulse rate and blood pressure were made on a small series of normal and diabetic subjects.

Epinephrin gave the usual rise of R.Q., heat production and blood sugar, as well as the well known subjective and circulatory changes in both normal and diabetic individuals. The R.Q. of patients with diabetes did not reach as high a figure as in any control case. The patients formed two groups according as to whether the increment of heat production was above or below that of the normal subjects. No parallelism between the behavior of blood sugar after epinephrin and severity of the disease could be made out.

The alveolar CO₂ tension, effective alveolar ventilation and dead space of a normal individual after epinephrin were calculated. The evidence from that experiment makes it quite doubtful whether the higher respiratory quotients after epinephrin indicate a relative increase in carbohydrate oxidation.

Insulin increased the R. Q. and heat production and greatly reduced the blood sugar in all experiments. In some cases the R.Q. diminished slightly during the first half hour after insulin. Pulse and blood pressure changes were slight and variable. Subjective symptoms were described. They were often more marked on the initial dose than on later injections irrespective of the level of blood sugar. Several normal persons had an extreme hypoglycemia without subjective sensations. As diabetic patients improved clinically, the effect of the same dosage of insulin caused a more immediate but smaller drop in percentage of blood sugar and the sugar started to increase in a shorter time after the injection. Subjects who showed a slight reaction to epinephrin tended to give a relatively marked response to insulin.

Injection of both insulin and epinephrin showed an antagonistic effect in their action on respiratory exchange, level of blood sugar and circulatory changes. The total result was the summation of the 2 factors. In the case of 2 diabetic patients epinephrin failed to terminate a condition of hypoglycemia which was brought on by insulin, the blood sugar percentage continuing to decrease after epinephrin was administered.

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THE RÔLE OF THE LIVER IN THE REMOVAL OF HEMOGLOBIN FROM THE BLOOD STREAM

SANFORD M. ROSENTHAL

Fellow National Research Council

From the Pharmacological Laboratory, the Johns Hopkins University

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While it is generally conceded that hemoglobin or its derivatives form the principal source of bile pigments and that the liver epithelium excretes the formed pigments, the actual steps involved in the transformation, the regulatory mechanism of hemoglobin destruction and bile pigment formation, and the chief site at which these changes take place, have not been firmly established. Virchow in 1847 (1) recovered hematoidin crystals from old hemorrhagic exudates and they were later identified with bilirubin by Jaffé (2) and numerous subsequent investigators. Stadelman (3) produced jaundice by administering hemolytic agents, and Minkowski and Naunyn (4) concluded from their experiments on fowls that such a jaundice could not be produced if the liver were extirpated. Their results were confirmed by Löwit (5) upon frogs and by Stern (6) upon pigeons. These experiments laid the foundation for the belief that hemoglobin was transformed into bile pigments in the liver only, and this view was strengthened by the demonstration that hemoglobin and hemin injections caused an immediate increase in the excretion of bile pigments, Tarchanoff (7) Stadelman (3), Brugsch and Yoshimoto (8), Brugsch and Kawashima (9), Gilbert, Chabrol and Bernard (10) Jones (11). A review of the subject of blood destruction has been written recently by Rous (12); Whipple (13) has published a review of bile pigment metabolism.

Whipple and Hooper have made an extensive study of hemoglobin and bile pigment metabolism, and their researches have

made certain phases of the older teachings untenable. They demonstrated clearly that hemoglobin could be transformed into bile pigments without the intervention of the liver (14) (15), and concluded from their experiments that this change took place as rapidly as normal in Eck fistula dogs and in Eck fistula dogs with the hepatic artery ligated, but their experiments were quantitative only in so far as the time of appearance of bile pigments in the blood and urine (14), (16). Furthermore in experiments upon bile fistula dogs they found that no quantitative relation existed between the amount of hemoglobin injected and the resulting increase in quantity of bile pigments excreted during the first six hours (17). Jones (11) has shown that the extra hepatic formation of bile pigments from hemoglobin can take place rapidly in man. McNee (18) in reporting the experiments of Minkowski and Naunyn, concluded that no *marked* icterus could occur following liver extirpation. Such evidence would serve to make uncertain the exact rôle played by the liver in hemoglobin catabolism, and since quantitative experiments are lacking, it was felt that the determination of the rate of disappearance of hemoglobin from the blood stream before and after ligation of the liver might help to establish the part that the liver plays in breaking down hemoglobin into its pigment derivatives.

METHOD

Total extirpation of the liver at one operation was so frequently attended by intravascular hemolysis that this procedure was abandoned in mammals; serial extirpation, as developed by Mann (19), might permit other organs partially to compensate for the resulting disturbance in pigment metabolism. Advantage was therefore taken of the existence in the rabbit of a main liver which comprises about 80 per cent of the total hepatic substance, which has independent vascular communications and which is entirely separate from the remaining lobe mass. Ponfick (20) has shown that removal of the main liver is attended by no ill effects, the lobe mass being entirely adequate for the maintenance of the portal circulation.

Hemoglobin solution was obtained by laking with distilled water the washed red cells of healthy rabbits kept as bleeders. This solution was freshly prepared for each experiment, and before injection it was made isotonic by the addition of sodium chloride. The hemoglobin solution was kept on ice for the twenty-four hours that elapsed from the determination of the normal curve of disappearance until the repetition of the test after the liver had been ligated. The strength of the hemoglobin solution was estimated by the method of Newcomer (21). As it was desirable to exclude the factor of renal elimination, the dosage employed did not exceed 60 mgm. of hemoglobin per kilo of body weight, since this amount has been established by Pearce Austin and Eisenberg (22) as the renal threshold for hemoglobin when injected intravenously. In the following experiments no hemoglobinuria occurred in the normal rabbits, or in those with the main liver ligated.

The technique carried out was as follows: under ether anesthesia for ten minutes the external jugular vein of a rabbit was exposed, and 3 or 4 cc. of blood drawn. This sample served as a check against any hemolysis which might result from the apparatus employed; the clear serum so obtained was also used in the preparation of a standard for colorimetric comparison. The calculated dose of isotonic hemoglobin solution was injected into an ear vein, and several samples of blood subsequently drawn over a period of four hours. The bloods were allowed to clot and the serum was obtained by centrifugation at high speed. A standard of known strength was now prepared from the hemoglobin solution, and the amount of hemoglobin in the unknown serum was determined by colorimetric comparison with it. On the following day the animal was again anesthetized for ten to fifteen minutes and a ligature was firmly placed around the portal vein, the hepatic artery, and the bile duct of the main liver; the small caudate lobe was also ligated if necessary. Fifteen minutes after ligation the control sample of blood was drawn, the same amount of hemoglobin solution again injected into an ear vein, and its rate of disappearance from the serum determined. Ligation of the main liver was performed upon

two control animals, and samples of blood taken over a period of four hours, in order to prove that no free hemoglobin was liberated into the circulation as a result of the operation itself.

RESULTS

Hemoglobin leaves the blood stream rather slowly. Lee and Whipple (23), upon a basis that none leaves the plasma within the first few minutes after it is injected intravenously, have utilized hemoglobin experimentally in the determination of plasma volume. They employed a uniform amount of 'laked corpuscular solution, containing approximately 1.44 gram of hemoglobin, and in 6 normal dogs studied the rate of departure during the first two hours that followed the injection; plasma obtained eighteen hours after the injection was found by them to be perfectly clear.

With a dosage of 22 to 60 mgm. of hemoglobin per kilo of body weight, the rate of disappearance from the blood serum in 11 normal rabbits, was studied. Irrespective of the dose when below 60 mgm. per kilo, practically complete removal occurred within four hours (fig. 1). In rabbit 1 however, intravascular hemolysis resulted from the injection, probably because in this experiment the hemoglobin solution was not rendered isotonic, and a slightly higher curve of disappearance resulted; the amount of free hemoglobin that was present in this experiment, as calculated by the early concentration in the serum, was approximately 80 mgm. per kilo of body weight.

After the normal curves had been obtained, the tests were repeated in 5 rabbits with ligation of the main liver (fig. 2). The amount of liver substance tied off averaged 78.8 per cent of the entire organ, and comprised 2.5 per cent of the total body weight of the animals. An analysis of the results (fig. 3) shows that 45 mgm. of hemoglobin per 100 cc. of serum or 43 per cent of the original concentration, was still present four hours after the injection, as contrasted with almost complete disappearance in the normal animals. The increased concentrations that were present immediately after the injection in the ligation experiments, were probably due to the restricted volume of circulating blood which resulted from the liver ligation.

Such experiments demonstrate the importance of the liver in hemoglobin metabolism, and show that rabbits with damaged livers cannot remove hemoglobin from the blood stream as quickly as can normal ones. It would appear from a quantitative standpoint that the transformation of hemoglobin into bile pigments cannot proceed as well in animals with damaged livers

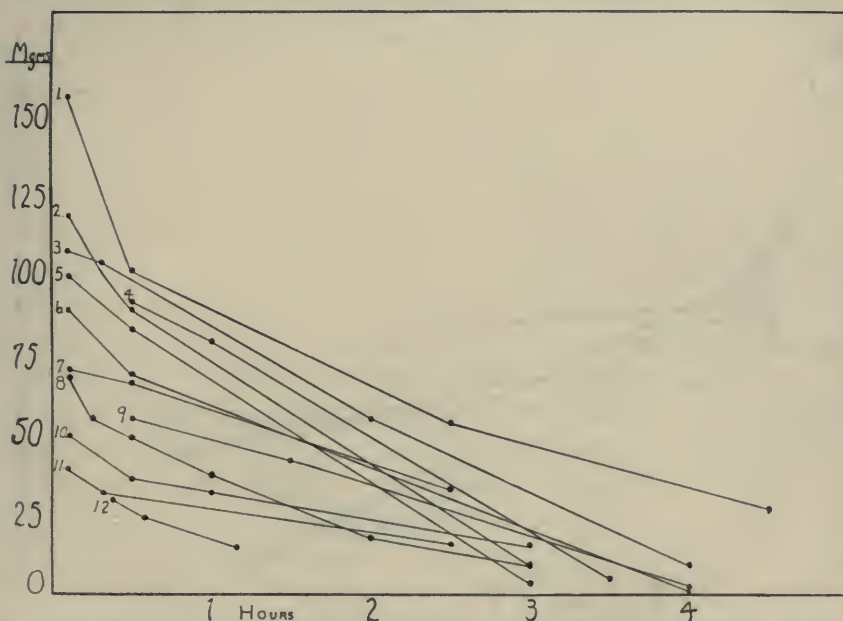


FIG. 1. DISAPPEARANCE OF HEMOGLOBIN FROM THE BLOOD STREAM IN NORMAL RABBITS

The amount of hemoglobin injected intravenously per kilo of body weight was as follows: Rabbit 1, 80 mgm., rabbit 2, 48 mgm., rabbit 3, 60 mgm., rabbit 4, 45 mgm., rabbit 5, 50 mgm., rabbit 6, 46 mgm., rabbit 7, 34 mgm., rabbit 8, 50 mgm., rabbit 9, 33 mgm., rabbit 10, 30 mgm., rabbit 11, 22 mgm., rabbit 12, 25 mgm.

as in normal animals but this conclusion should be verified by a quantitative study of the bilirubinemia that results from hemoglobin injections in normal animals and in those with damaged livers.

In the above ligation experiments, approximately one-fifth of the liver was unligated and functioning, and in all likelihood

compensated to a considerable degree for the loss of function of the ligated four-fifths. Estimations of the part played by the entire liver in the removal of hemoglobin from the blood would necessarily be too low, according to the degree of compensation that took place in the functioning lobe mass. In order to demonstrate however, that main lobe ligation as described

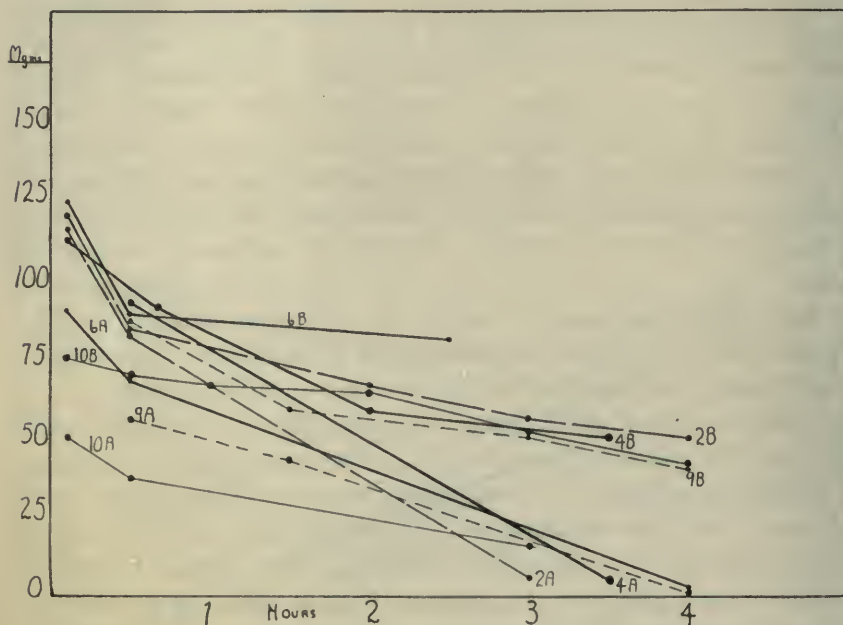


FIG. 2. RATE OF DEPARTURE OF HEMOGLOBIN FROM THE BLOOD STREAM IN RABBITS BEFORE AND AFTER LIGATION OF THE MAIN LIVER

The per cent of total liver substance ligated was as follows: Rabbit 2, 70 per cent; rabbit 4, 81 per cent; rabbit 6, 78 per cent; rabbit 9, 80 per cent; rabbit 10, 84 per cent.

above, does cause a severe impairment of liver function, tests were carried out with phenoltetrachlorphthalein, using the method I devised for the quantitative determination of hepatic function with this dye (24), (25). These results will be published fully in another paper, but it is seen from figure 4 that main lobe ligation causes a severe damage to hepatic function. This degree of retention of dye has been found to represent ap-

proximately 58 per cent of the tetrachlorophthalein retention obtained with complete removal of the rabbit's liver. As measured by this test, therefore, ligation of the main liver causes an impairment of 58 per cent of function, and should this hold true for the removal of hemoglobin from the blood stream, then removal of the entire liver would result in a retention at the 4 hour period of 74 per cent of the amount of hemoglobin injected.

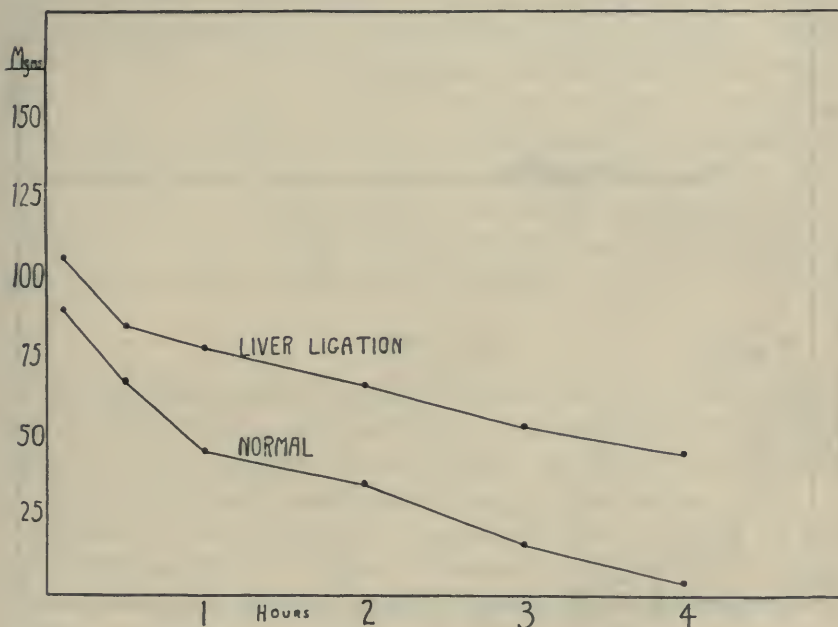


FIG. 3. DEPARTURE OF HEMOGLOBIN FROM BLOOD STREAM BEFORE AND AFTER LIGATION OF THE MAIN LIVER

Composite arranged from figure 2

Pearce, Austin and Eisenberg studied in normal and splenectomized dogs the hemoglobinuria and choluria that followed hemoglobin injections (22); their conclusions, based upon the appearance time of bile pigments, and the quantity of hemoglobin excreted in the urine, were that splenectomy did not retard the departure of hemoglobin from the blood stream, or interfere with its transformation into bile pigments. Gilbert, Chabrol and Bernard (10) came to similar conclusions from their experiments

upon bile pigment excretion following hemoglobin injections into the veins of normal and splenectomized dogs. These experiments are especially significant in view of the positive findings that I have obtained after liver ligation. Austin and Pepper (26) found that when hemoglobin was injected into the portal circulation it caused less marked hemoglobinuria and more persistent choluria than when similar injections were made into

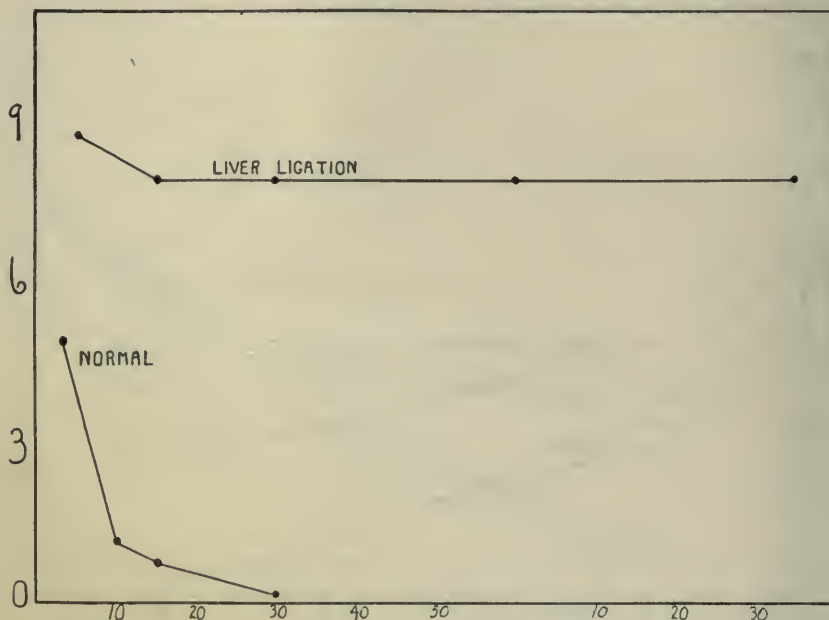


FIG. 4. PHENOLTETRACHLOROPHTHALEIN LIVER FUNCTION TEST IN NORMAL RABBITS AND IN THOSE WITH THE MAIN LIVER LIGATED

Curves show rate of disappearance of the dye from the blood stream following the injection of 5 mgm. per kilo of body weight.

the femoral vein, and they ascribe these findings to the specialized ability of the liver to take hemoglobin out of the blood stream and transform it into bile pigments.

It is apparent from the above experiments that the disappearance of hemoglobin from the blood stream could be utilized to test hepatic function. Barlocco (27) injected hemoglobin intravenously, and determined the subsequent degree of bili-

rubinemia, as an index of liver function, and Maragliano (28) speaks favorably of this procedure. Sellards and Minot (29) estimated the amount of hemoglobinuria that occurs in man after hemoglobin injections, and reported abnormal findings in pernicious anemia, and in 2 cases of liver disease. The rate of disappearance of hemoglobin from the blood stream should be more sensitive as a test than its excretion in the urine, because of the exclusion of the factor of renal disease, and because of the high renal threshold that normally exists for hemoglobin. However, the slower rate of departure from the blood, the wider physiological variations and the increased liability to technical error make hemoglobin far less sensitive and less reliable than phenoltetrachlorophthalein similarly employed as a test for liver function; this is demonstrated in the above experiments by a comparison of the results obtained with these two substances after ligation of the main liver in rabbits.

SUMMARY

The disappearance of hemoglobin from the blood serum following its injection intravenously has been studied in 12 normal rabbits. In a dosage of 22 to 60 mgm. per kilo of body weight there was practically complete removal in four hours.

Ligation of the main liver, comprising 78.8 per cent of the total liver substance, caused a marked delay in this rate of departure of hemoglobin from the blood. In 5 rabbits with the main liver ligated, an average of 43 per cent of the amount injected was present in the serum 4 hours after injection.

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THE FREE SUGAR CONTENT OF THE LIVER AND ITS RELATION TO GLYCOGENSYNTHESIS AND GLYCOGENOLYSIS

CARL F. CORI, G. T. CORI AND G. W. PUCHER¹

From the State Institute for the Study of Malignant Disease, H. R. Gaylord, Director

Received for publication June 4, 1923

The first step in the utilization of sugar is undoubtedly its penetration into the cells. There are now available two main processes for its disposal: first, oxidation and, second, synthesis to glycogen. For this latter phase, the liver is the chief organ. Since ingestion of sugar causes first a temporary hyperglycemia and then glycogensynthesis it was of importance to know how this increased blood sugar influenced the free liver sugar. This type of experiment gave us information concerning the conditions under which sugar is taken up by the liver. The output of sugar by the liver on the other hand and its relation to the free liver and the blood sugar was studied by experimental glycogenolysis.

Our paper deals with three questions: (a) Influence of glucose ingestion on the blood sugar, free sugar and glycogen content of the liver; (b) influence of iletin on the blood sugar, free sugar and glycogen content of the liver; (c) influence of adrenalin on the blood sugar, free sugar, and glycogen content of the liver.

In 1917 Palmer (1) studied the concentration of dextrose in the tissues of normal and diabetic animals. A review of the literature is found in this paper. The following conclusions of Palmer are related to our problems:

1. The concentration of dextrose in the tissues varies directly with the degree of hyperglycemia.

¹ From the Buffalo General Hospital.

2. The concentration of dextrose in tissues is invariably lower than the blood sugar except in the liver where the higher concentrations found may be explained by rapid glycogenolysis.

SURGICAL METHODS

In order to study the problems outlined above it was first necessary to devise a method² whereby pieces of liver could be frequently removed from the live animal under conditions that would not produce an appreciable hyperglycemia. For this purpose it was necessary to have a permanent opening in the abdominal wall through which the liver could be reached without applying narcosis or undue pain. This was accomplished by a preliminary operation by which a specially constructed apparatus, the details of which are shown in figure 1, was sewn in. The window constructed of silver plated metal, consists of two parts. The lower part which is sewn into the abdominal wall has an outside diameter of 4.5 cm. and an inside diameter of 2.5 cm. The height is 1.6 cm. The second part consists of a cover which can be screwed on to the lower part so as to close tightly the opening in the abdominal wall (fig. 1). The preliminary operation was performed under urethane-ether narcosis under aseptic precautions. A small incision was made in the middle line beginning at the end of the sternum. The lower part of the processus xiphoides was resected after the peritoneal cavity had been opened. The window was then slipped into the opening and attached with six stitches of catgut, three on each side of the lower edge and three on each side of the upper edge of the incision. The needle was first introduced through the whole muscular layer and peritoneum then through the appropriate hole of the window, and the ligature tied in such a way as to tightly fix the tissue onto the small circular groove at the base plate of the window. The complete protection of the peritoneal cavity was secured by a purse string suture of the skin. After the completion of the operation the cover is screwed on.

² This method was presented before the Western New York Branch of the Society for Experimental Biology and Medicine. Proceedings, vol. 20, no. 7, p. 409.

Twenty-four hours later the animal has recovered from the operation and will consume food. From then on, it may be used for experimentation. However, in this work the animals were not used until three to five days after the operation. No disturbances in the motility of the bowels were observed during the time that the window was in place. No efforts were made to determine how long the animal could exist under these conditions since after the completion of our experiments the window was removed.

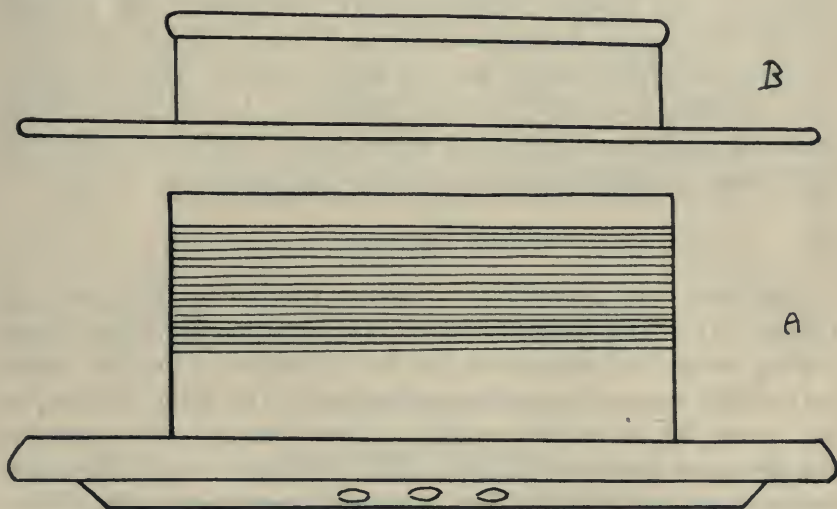


FIG. 1. ABDOMINAL WINDOW

(A) Lower part, (B) cover. Three times natural size.

By screwing off the cover nearly every lobe of the liver can be reached and drawn through the opening of the window. At proper intervals (one-half to one hour) pieces of liver of the desired size were cut off by a pair of slightly heated scissors, or the bleeding was stopped by application of Squibbs Thromboplastin, and a small tamponade. During this procedure the animal showed no evidence of pain or excitement. Six to ten pieces of liver of about 1 gram could be easily obtained during one experiment. A further advantage of this method is that

solutions can be directly injected into the stomach, and that two experiments can be performed on the same animal on subsequent days.

CHEMICAL METHODS

Blood sugar

Blood was drawn from the ear vein and the sugar therein determined by a slight modification of the Folin and Wu method. Instead of using the prescribed sugar tubes of Folin-Wu, re-oxidation was entirely prevented by overlaying the solution during the heating period with about 2 cc. of toluene or xylene. By this procedure one can use the sugar tubes of Myer-Benedict thus enabling a variety of final dilutions. No higher standard than 0.2 mgm. was ever used since it has been found that the high sugar concentrations do not give proportionate values.

Free liver sugar

This was determined by a method similar to that used by Palmer (1). The weighed liver was immediately thrown into boiling water and extracted for five hours on a boiling water baths with successive portions of water. The final volume being about 50 times the weight of the sample. The proteins and glycogen were precipitated with colloidal iron and the sugar determined by the Folin-Wu or Bertrand methods. Extraction of the protein free solution of the liver sugar with Lloyd's reagent was omitted since it did not alter appreciably the final sugar values. It was also found that small and large pieces of liver when extracted separately gave the same sugar value.

Glycogen

Owing to the small amounts of liver available Pfügers' method could not be applied. The Glycogen was evaluated from the difference between the total carbohydrate and the free sugar content of the liver. The total carbohydrates were determined by a method similar to that of O. Loewi's (2) for the estimation of the total carbohydrates of the muscles. Five-tenths to 1 gram

of liver was cut in small pieces and introduced into a 25-cc. volumetric flask containing 10 cc. of 2.2 per cent HCl. These were then heated in a boiling water bath for five hours the particles of the liver being thoroughly macerated by means of a glass rod. It was found that heating for five hours was sufficient for complete hydrolysis of the glycogen. The flasks were then cooled, the solution made almost neutral with KOH and the proteins precipitated with 30 per cent mercury acetate. After diluting to the mark, mixing and filtering the mercury was removed by H_2S , the excess gas expelled by a current of air and the sugar in an aliquot part determined by the Bertrand method.

For all experiments in which the window method was applied rabbits were used. In certain of the experiments, series of twenty-four hour fasting guinea-pigs were employed. These were killed by a blow on the head at various time intervals after ingestion of glucose or injection of adrenalin. The whole liver was immediately removed and analyzed for free sugar and glycogen as previously described. The average time that elapsed between stunning and introduction of the liver into the boiling water was three minutes.

EXPERIMENTAL DATA

a. Glucose ingestion

The effect of glucose ingestion on the blood sugar, free liver sugar and glycogen content of the liver are presented in the following tables. It will be noted that the quantities of glucose fed were far beneath the limits of assimilability of rabbits. Mendel (3) determined this to be 13 grams per kilogram, whereas in our experiments only 2 to 2.5 grams glucose per kilogram were fed.

These experiments show that with the increase of the blood sugar there is always a decided rise in the free liver sugar. These data also show a simultaneous increase of the glycogen.

TABLE 1

Rabbit 4; operated April 2, 1923; weight, 2000 grams; day of experiment, April 5, 1923; 24 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR
	<i>per cent</i>	<i>per cent</i>
9:50	0.131	0.319
10:05	5 grams of glucose injected into the stomach	
10:35	0.408	0.604
11:09	0.226	0.32
12:03	0.158	0.35

TABLE 2

Rabbit 6; operated April 13, 1923; weight, 1700 grams; day of experiment April 16, 1923; 24 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR	GLYCOGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9:50	0.120	0.354	1.5
10:00	5 grams glucose injected into the stomach		
10:30	0.149	0.43	1.8
11:03	0.175	0.49	2.0
12:03	0.226	0.45	2.48

TABLE 3

Rabbit 9; operated April 23, 1923; weight, 3380 grams; day of experiment, April 26, 1923; 24 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR	GLYCOGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9:40	0.113	0.378	2.72
9:50	8.5 grams glucose injected subcutaneously		
10:48	0.206	0.415	2.84
11:50	0.256	0.520	2.54
12:47	0.330	0.66	3.73

b. Glucose ingestion plus iletin

The same experiments as in A were repeated on rabbits under the influence of iletin (Lilly). The amount of iletin injected subcutaneously was large enough to cause a lowering of the blood sugar even though glucose was simultaneously ingested.

Experiment in table 9 in which a larger amount of glucose was fed is the only exception. In order to minimize the individual difference of the animals, several experiments of group A and B were performed on the same rabbit (tables 1 and 6, 2 and 8, 4 and 10).

TABLE 4

Rabbit 11; operated May 1, 1923; weight, 8000 grams; day of experiment May 4, 1923; 35 hours starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR	GLYCOGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9:35	0.105	0.354	1.05
9:45	7.5 grams sugar injected into the stomach		
10:45	0.166	0.46	1.51
11:45	0.213	0.441	1.5
12:45	0.209	0.413	1.3

TABLE 5

Series of 24 hour fasting guinea-pigs killed at different time intervals. The food, 2 grams of dextrose and 5 grams of bread, was consumed within five minutes

TIME AFTER INGESTION OF 12 GRAMS GLUCOSE	BLOOD SUGAR, MG. PER 100 CC.	FREE SUGAR IN THE LIVER, MG. PER 100 GRAMS	LIVER GLYCOGEN, GRAMS PER 100 GRAMS	FREE LIVER SUGAR MINUS BLOOD SUGAR
<i>Minutes</i>				
30	314	658	1.40	344
60	300	555	1.03	255
90	300	670	1.48	370
120	202	602	2.24	400
150	233	519	4.24	316
180	316	617	3.60	301
180	223	666	4.95	443
210	191	357	3.38	166

It will be seen that iletin always causes a decrease of the free liver sugar. This is true even though the blood sugar may remain above its normal level as shown in experiment in table 9 where larger doses of glucose were administered. In spite of the fact that both blood sugar and liver sugar are decreased under iletin, yet there is a continual building up of the liver glycogen.

TABLE 6

Rabbit 4; operated April 2, 1923; weight, 2000 grams; day of experiment April 6, 1923; 24 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR
	<i>per cent</i>	<i>per cent</i>
10:00	2 units iletin subcutaneously	
10:15	0.112	0.322
10:25	5 grams glucose injected into the stomach	
10:27	2 units iletin subcutaneously	
11:00	0.098	0.29
11:10	2 units iletin subcutaneously	
12:00	0.092	0.34
12:30	0.093	0.26

TABLE 7

Rabbit 5; operated April 9, 1923; weight, 1900 grams; day of experiment April 11, 1923; 48 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR	GLYCOGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9:35	2 units iletin subcutaneously		
9:55	0.072	0.183	0.86
10:07	5 grams glucose injected into the stomach		
10:08	2 units iletin		
10:37	0.08	0.277	1.06
11:06	0.077	0.234	1.59
12:03	0.062	0.271	1.70
2:00		Convulsions	

TABLE 8

Rabbit 6; operated April 13, 1923; weight, 1700 grams; day of experiment April 17, 1923; 12 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR	GLYCOGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
11:55	2 units iletin subcutaneously		
12:15	0.081	0.300	1.43
12:25	5 grams glucose injected into the stomach		
12:35	2 units iletin		
1:10	0.077	0.28	Lost
1:25	0.074	0.32	1.8
2:30	0.072	0.24	1.9

TABLE 9

Rabbit 7; operated April 17, 1923; weight 2800 grams; day of experiment April 20, 1923; 19 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR	GLYCOGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9:35	0.128	0.318	0.72
9:45	2 units iletin subcutaneously		
10:05	10 grams sugar injected into the stomach		
10:20	2 units iletin subcutaneously		
10:35	0.212		
11:05	0.248	0.32	1.87
11:40	2 units iletin subcutaneously		
12:10	0.190	0.289	3.08
1:10	Lost	0.295	3.47
4:00		Convulsions	

TABLE 10

Rabbit 11; operated May 1, 1923; weight, 3000 grams; day of experiment May 5, 1923; 24 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR	GLYCOGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9:25	2 units iletin subcutaneously		
9:40	0.101	0.339	0.473
9:55	7.5 grams sugar injected into the stomach		
10:25	2 units iletin subcutaneously		
10:55	0.06	0.295	1.12
11:50	0.068	0.282	0.84
12:55	0.071	0.233	0.618

TABLE 11

Rabbit 12; operated May 20, 1923; weight, 3220 grams; day of experiment May 23, 1923; 24 hour starving

TIME	BLOOD SUGAR	FREE LIVER SUGAR	GLYCOGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
9:30	2 units iletin subcutaneously		
9:50	0.091	0.35	1.35
10:00	8 grams sugar injected into the stomach		
10:30	2 units iletin subcutaneously		
10:55	0.074	0.278	1.70
12:00	0.079	0.202	1.75
1:00	0.068	0.32	1.62

c. Adrenalin

In order to study free liver sugar during glycogenolysis and its rate of diffusion into the blood stream adrenalin was subcutaneously injected into rabbits and guinea-pigs. The data

TABLE 12

Rabbit 1; operated March 10, 1923; day of experiment March 14, 1923; 24 hour fasting

TIME	BLOOD SUGAR	FREE LIVER SUGAR
	<i>per cent</i>	<i>per cent</i>
10:30	0.137	0.215
10:33	1 mgm. adrenaline subcutaneously	
11:00	0.286	0.440
11:35	0.384	1.68
12:30	0.510	0.716
1:30	0.545	0.767
5:10	0.240	0.364

TABLE 13

Series of 24 hour fasting guinea-pigs killed at different time intervals after the injection of 0.5 mgm. adrenaline

TIME AFTER INJECTION OF 0.5 MGm. ADRENALIN	BLOOD SUGAR, MGm. PER 100 CC.	FREE SUGAR IN THE LIVER, MGm. PER 100 GRAMS	LIVER GLYCOGEN, GRAMS PER 100 GRAMS	LIVER SUGAR MINUS BLOOD SUGAR
<i>minutes</i>				
10	159	495	4.00	336
20	190	548	1.35	358
30	288	700	2.03	412
30	279	865	3.04	586
30	158	651	2.18	493
40	264	594	1.82	330
60	244	560		316
60	168	470		302
90	356	306	3.08	-50
Control	104	363	4.81	259

were obtained as described under Methods and are reported in detail in tables 12 and 13.

When relatively large doses of adrenalin are injected in well nourished animals, there is an instantaneous liberation of large quantities of free sugar in the liver which reaches its maximum

amount in guinea pigs in about thirty minutes, and in rabbits in about one hour. The free sugar may attain values as high as 1.7 per cent. After this period there is already a precipitous decrease of the free liver sugar. The blood sugar, on the other hand, rises slowly and reaches its maximum value much later than the free liver sugar. This indicates that the diffusion of the free sugar into the blood stream is a gradual process.

DISCUSSION

In all our experiments the free liver sugar was higher than the blood sugar. The values for the free sugar found in rabbits previously starved from twenty-four to forty-eight hours are 0.3 to 0.35 per cent. These values are slightly larger than those recorded by Pavy (4) and Palmer (1). The influence of the blood content of the liver may be regarded negligible as regards the free sugar content of that organ. This can be seen from a simple calculation. Since, before analysis the blood was pressed out, probably not more than 10 to 15 per cent of blood remains in the section analyzed. If the blood sugar was 0.2 per cent an error of about plus 20 mgm. could be introduced. Since our conclusions are always drawn from larger differences, the factor was neglected in the calculations of our data.

Our experiments clearly show that simultaneously with the glycogensynthesis, there is always an increase of the free liver sugar. This means that glycogen is only built up under physiological conditions, at a free sugar level higher than that of a starving animal. These facts lead us to believe that glycogen synthesis is dependent upon the free sugar level in the liver. In other words, whether the reaction: $\text{glucose} \rightleftharpoons \text{glycogen}$, goes to the right or to the left is dependent, in part at least, upon the level of the free liver sugar. If the free sugar is high (above that of a starving animal) glycogen synthesis takes place. This is, of course, the phenomenon which occurs during the absorption of glucose. The experiment of Palmer's (l.c., p. 88) where he injected glucose intravenously into glycogen free rabbit, is in complete harmony with this data. With iletin however, one obtains not only a reduction of the blood sugar, but also a

decrease of the free liver sugar. Nevertheless, under these abnormal conditions we found glycogen synthesis occurring at about the same velocity and intensity as in those animals with a high free liver sugar. This indicates that the free sugar level from which glycogen synthesis takes place has been changed by iletin. How iletin causes this phenomenon is not yet clear, but we believe that ferment studies will show some light upon this question.

McLeod and his co-workers (5) have recently published a paper in which they suggest several mechanisms for the hypoglycemic action of insulin. Iletin may cause a sugar vacuum in the cells. "The causes for the setting up by insulin of a sugar vacuum in the cells may be either that it stimulates combustion of the sugar or that it condenses it into glycogen or reduces it to fatty acids." Which of these possibilities is actually involved has not yet been determined. The data in favor of the theory that iletin involves glycogensynthesis and thus lowers the blood sugar has been obtained by McLeod and his co-workers (5), using diabetic dogs over a long period of time. No experimental data are available on normal animals. Our data from normal rabbits show that iletin disturbs the mechanism of blood sugar regulation, in part at least, through glycogensynthesis. The other factors possibly involved have not yet been studied.

Palmer (l.c., p. 92) reports 3 experiments on the effect of adrenalin on the dextrose concentration of the tissues of dogs. Two of these experiments show two hours after the adrenalin injection a free liver sugar of 0.52 and 0.51 per cent with a blood sugar of 0.26 and 0.24 per cent. Our experiments agree with this data and demonstrate the instantaneous action of adrenalin and the relatively slow diffusion into the blood stream of the sugar set free in the liver.

CONCLUSIONS

1. During the ingestion of glucose by rabbits and guinea-pigs glycogensynthesis takes place from a free sugar level above that of a starving animal.

2. Iletin causes glycogen synthesis during the ingestion of glucose even though the blood sugar and free liver sugar is below that of a starved animal.

3. Adrenalin immediately stimulates glycogenolysis setting free the maximum amount of sugar in the liver in from thirty to sixty minutes. The sugar set free in the liver is only slowly put into the blood stream. The highest blood sugar is at a point where the free liver sugar has already begun to decrease.

We wish to thank Miss Hilda Goltz for carrying out the glycogen determinations and to Miss Cora Geisler for surgical assistance.

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The first of these is the fact that the United States is a young nation. It is only about 150 years old, and its history is therefore a very recent one. This is in contrast to the history of the European countries, which are much older and have a much longer and more varied history. The second fact is that the United States is a large country. It covers a vast area of land, and its population is very large. This is in contrast to the European countries, which are much smaller and have a much smaller population. The third fact is that the United States is a diverse country. It is made up of many different peoples, and its culture is a mixture of many different influences. This is in contrast to the European countries, which are much more homogeneous in their population and culture.

The fourth fact is that the United States is a free country. It is a country in which the people are free to express their opinions and to follow their own paths. This is in contrast to the European countries, which are much more authoritarian and have a much longer history of oppression. The fifth fact is that the United States is a country of opportunity. It is a country in which the people have the chance to improve their lives and to achieve their dreams. This is in contrast to the European countries, which are much more stagnant and have a much longer history of poverty and hardship.

The sixth fact is that the United States is a country of progress. It is a country in which the people are always looking for new ways to improve their lives and to make the world a better place. This is in contrast to the European countries, which are much more conservative and have a much longer history of resistance to change. The seventh fact is that the United States is a country of hope. It is a country in which the people believe that the future is bright and that they can make a difference in the world. This is in contrast to the European countries, which are much more pessimistic and have a much longer history of despair.

The eighth fact is that the United States is a country of peace. It is a country in which the people are committed to peace and to the resolution of conflicts through peaceful means. This is in contrast to the European countries, which are much more prone to war and to the use of force.

NOTES ON THE PHARMACOLOGY AND THERAPEUTICS OF OIL OF CHENOPODIUM AND INVESTIGATIONS ON THE ANTHELMINTIC VALUE OF ITS COMPONENTS¹

DANIEL M. MOLLOY

Managua, Nicaragua

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Chenopodium is one of the most widely used anthelmintics known to medicine. Most Southerners remember vividly the molasses-candy—Jerusalem-oak mixture which their mothers gave them religiously at the beginning of spring. "Herb doctors" have used it from time immemorial, and it is a household remedy used throughout the entire Western Hemisphere. Infusions of the leaves and seed of "apazote" were probably used as a vermicide by the Aztecs centuries before the Spanish conquest. The essential oil distilled from the seeds was first introduced into therapeutics as an ascaricide. It was not used to any considerable extent as an uncinaricide, however, until Schüffner and Baermann began to use it for this purpose on the rubber estates of eastern Sumatra in 1915. Toward the end of 1915 Schüffner (1) reported having used the oil in the treatment of more than forty thousand cases of hookworm disease, without after effects, and with results far superior to those which had previously been obtained with thymol. When these results became known, oil of chenopodium was rapidly adopted as the standard treatment for hookworm disease and it now undoubtedly occupies first place in the treatment of this tropical and subtropical scourge.

The physiologic action of the drug is well known, and nothing of great importance has been added to the sum total of our knowl-

¹ The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Board of the Rockefeller Foundation.

edge of the effect of small doses of the drug on the human organism within recent years. Little was known of the effects of large doses, however, until reports of untoward symptoms following its administration as an uncinaricide began to appear. The normal dose of the drug as an ascaricide rarely produced toxic symptoms. When the dose was greatly increased, as was found necessary for expelling uncinarias, alarming symptoms, and sometimes death, not infrequently resulted. Brüning (2) was the first to call attention to its toxicity after a series of tests on different animals. Salant and Nelson (3) later repeated these experiments and showed that the drug is a strong gastrointestinal irritant. Hall (4) noted that the injury to the patient probably depends on the rate of absorption of the drug and that two factors are to be considered in this connection: First the immediate local effect on the gastrointestinal tract, and second, the more remote irritant and toxic effects on the circulatory, respiratory, excretory, and nervous systems. In cases of poisoning with the drug which have come under our observation in Nicaragua, where the drug has been used almost exclusively in the treatment of hookworm disease for more than six years, the neurotoxic symptoms have been most prominent. Circulatory and respiratory disturbances have been next in importance, and least important of all are the irritant effects of the drug in the digestive tract. That the action of the drug is not confined to its effect on the parasitic organisms against which it is directed, and that it may exert the effect of a strong local and systemic poison, is now well established, and must be taken into account when employing the drug on a large scale in campaigns for the reduction of hookworm disease. Salant (5) called special attention to the effect of the nutritional and diet conditions on the toxicity of the oil, and states that fasting or poorly nourished animals succumbed to much smaller doses of the drug, while animals which had been given a diet rich in carbohydrates for several days before the administration of the oil developed noticeably greater resistance to its toxic effects.

That the nutritional condition of the patient exerts a marked influence on the toxic effects of the drug there can be little doubt.

This has been borne out in our experience with more than two hundred thousand treatments given under field conditions. Three children in extreme stages of inanition succumbed to minimum doses of the drug, while two well-nourished adults survived ponderous doses administered by mistake. One of these, an American marine of the Legation Guard in Managua, survived a dose of 160 minims which was administered by a hospital attendant who misread *mins.* for *mils.* and thus administered a dose approximately 16 times greater than the one prescribed. With these experiences in mind, we have greatly decreased the number of serious cases of poisoning with the drug by either refusing to treat all cases suffering from extreme inanition, or postponing the treatment until the nutritional condition could be improved. From our experience it would also appear that a diet rich in carbohydrates tends to protect the individual against the toxic effects of the drug, and we now prescribe a carbohydrate diet when preparing debilitated patients for the treatment. Salant also found that the resistance of animals was noticeably increased when the administration of the oil was preceded by a sufficient quantity of a fatty oil, such as olive oil, cocoanut oil, or castor oil. While we have not been able confirm his findings completely, we invariably administer the drug in castor oil and precede the dose with castor oil when treating greatly debilitated individuals or very young children, and our experience leads us to believe that castor oil exerts a decided protective effect.

Repeated efforts have been made to find some vehicle for the drug which would hasten its passage through the intestinal tract without lessening its anthelmintic potency, but none of these efforts have met with success. Hall and Foster (6) combined the drug with liquid petrolatum, but found that the combination was not a success, the chenopodium losing its potency. Later Hall and Hamilton (7) conducted some experiments on the solubility of oil of chenopodium in liquid petrolatum which led to an investigation of the distillation products of the oil. When a mixture of chenopodium oil and liquid petrolatum was allowed to stand, a small amount of thick, dark brown material separated out at the bottom. An investigation of the anthelmintic prop-

erties of these components suggested that the drug might be divided into separate components by fractional distillation, thus isolating the active principle of the drug.

Their findings were distinctly at variance with those of Schimmel and Company (8) and Nelson (9) both of whom had separated from oil of chenopodium a considerable portion, with a distinct boiling-point, to which they had given the name ascaridole; while Hall and Hamilton were unable to divide the drug into distinct components by fractional distillation and concluded that the oil is composed of a "mixture of constituents of an undetermined character." They state, however, that "the first distillate is very light colored, almost water-white. The distillate becomes progressively darker as it distills at higher temperatures and soon acquires nearly the color of the original oil." They further state that "the first distillate soon acquires nearly the odor of the original oil, while the last distillate and that remaining in the flask has an odor resembling turpentine." Tests of the distillation products for anthelmintic properties gave some surprising results, since they would seem to have established a pharmacologic paradox. They state that "tests of the distillation products obtained indicated that the greatest anthelmintic efficacy resides in the lighter fraction of the oil, the efficacy suffering a diminution as the heavier fractions are used," and that "the heavier fractions have a well-marked action as gastrointestinal irritants," reaching the conclusion that oil of chenopodium should be redistilled "to eliminate a fraction which has less anthelmintic value and more irritant and toxic properties than the lighter fraction." Should their findings be confirmed, such a procedure would undoubtedly add greatly to the value of the drug. Nelson, however, questions the accuracy of their findings in a later paper (10) and states that they either "did not have a true oil of chenopodium, or, as seems more probable, allowed the oil to become overheated in the bath, thus causing decomposition and loss of a part of the ascaridole."

Impressed with the importance of the question raised by Hall and Hamilton, the writer obtained a quantity of the distillation products of oil of chenopodium from the Essential Oils Labora-

tory of the Drug Division, Bureau of Chemistry, Washington, D. C., for testing the treatment of human hookworm infections. In this connection, the writer wishes to thank Dr. E. K. Nelson, Chief of the Division, who personally prepared the distillation products for the tests.

Nelson (10) states that during the distillation of the lighter (terpene) fraction a bath temperature of 80° was found sufficient to maintain an even distillation and that a temperature of 115° was sufficient to maintain an even distillation of the heavier (ascaridole) fraction, and that the distillations were carried at 3 to 6 mm. pressure. The terpene fraction constitutes about 40 per cent of the whole oil, while the ascaridole fraction constitutes approximately 57 per cent, the remaining 3 per cent representing the distillation residue.

The terpene fraction is a "light-colored, almost water-white" liquid which has a distinct turpentine-like odor with a faint trace of the odor of the original oil. The color of the fraction has not changed during the two years that it has been in Nicaragua. The ascaridole fraction has nearly the color of the original oil and possesses the characteristic pungent odor of the whole oil.

Since Hall and Hamilton had found that "the greatest anthelmintic efficacy resides in the lighter fraction of the oil" (notwithstanding the fact that the heavier fraction in our possession more nearly resembles the whole oil in color, odor, taste, etc.), we decided to give a series of treatments with equal quantities of the two fractions, following these trial treatments ten days later with a test treatment of the whole oil. All of the tests described in this paper were made in the Nicaraguan Penitentiary and thanks are due to the warden and officials of the Penitentiary, as well as to the prisoners, who cheerfully submitted to the test treatments. These prisoners were selected at random after determining that they harbored hookworms by microscopical examination of the feces.

The results of this first series of trial treatments with the two fractions of the drug, as compared with the results obtained in both instances on administering a test treatment of the whole oil ten days later, will be given in two tables.

TABLE 1

Trial treatments with 1.00 mil of the terpene fraction, followed by a test treatment of 2.00 mils of the whole oil ten days later

CASE NUMBER	WORMS HARBORED	WORMS REMOVED BY TRIAL TREATMENT	WORMS REMOVED BY TEST TREATMENT	PERCENTAGE OF WORMS REMOVED BY TRIAL TREATMENT
1	150		150	
2	100		100	
3	201		201	
4	60		60	
5	200		200	
6	51		51	
7	172	1	171	00.06
8	210		210	
9	203		203	
10	101		101	
11	200		200	
Total.....	1,648	1	1,647	

TABLE 2

Trial treatments with 1.00 mil of the ascaridole fraction, followed by a test treatment of 2.00 mils of the whole oil ten days later

CASE NUMBER	WORMS REMOVED	WORMS REMOVED BY TRIAL TREATMENT	WORMS REMOVED BY TEST TREATMENT	PERCENTAGE OF WORMS REMOVED BY TRIAL TREATMENT
1	12	10	2	83.33
2	9	7	2	77.77
3	11	9	2	81.81
4	17	15	2	88.23
5	12	11	1	91.66
6	18	16	2	88.88
7	6	4	2	66.66
8	37	35	2	94.58
9	11	10	1	90.90
10	29	27	2	93.10
11	9	8	1	88.88
12	3	2	1	66.66
13	3	1	2	33.33
14	16	15	1	92.50
15	18	16	2	88.88
16	12	5	7	41.66
17	9	8	1	88.88
18	4	3	1	75.00
Total.....	236	202	34	85.59

Since the results of this series of treatments were not entirely conclusive, on account of the small dose used, another series of 14 trial treatments with 2.00 mls of the terpene fraction was given, followed ten days later by a test treatment of 2.00 mls of oil of chenopodium. The results of these trial treatments were almost identical with those of the former series, only 8 hookworms being expelled by the 14 trial treatments, while the test treatments expelled a total of 1979 worms. The results of these two series of trial treatments with the terpene fraction of the oil demonstrated conclusively that the lighter fraction of the oil possesses practically no anthelmintic properties. A series of trial treatments with 1.00 mil of the ascaridole fraction was then given with the results set forth in table 2.

In view of the fact that these two series of treatments demonstrated conclusively that the anthelmintic efficacy resides in the heavier fraction of the oil, rather than in the lighter fraction, as Hall and Hamilton found, a series of treatments was arranged to test the efficacy of 1.20 mls of ascaridole (60 per cent of the normal dose of the whole oil). Table 3 gives the results of this series of trial treatments.

From table 3 it will be seen that a dose of ascaridole which corresponds, to 60 per cent of the normal dose of the whole oil—this being the proportion of ascaridole distilled from the whole oil—removes, on the average, 98 per cent of the worms harbored by the individual treated. In no instance did the lighter fraction remove more than two or three worms, even when administered in doses almost three times as great as its proportionate dose (the terpenes constitute 40 per cent of the whole oil, hence the normal dose should be 40 per cent of the dose of the whole oil, or 0.80 mls).

Accurate records were kept of all symptoms which occurred after the administration of the trial treatments and these were compared with those produced by a normal dose of oil of chenopodium. Table 4 gives the symptoms observed.

From table 4 it will be seen that the symptoms produced by 1.20 mls of ascaridole are almost identical with those produced by 2.00 mls of the whole oil. This is in entire accord with the

anthelmintic effects produced, ascaridole being slightly more efficacious than the whole oil when given in doses which correspond to 60 per cent of the normal dose of the whole oil (2.00 mls). (Unfortunately we were unable to carry out a series of treatments which was planned for the purpose of comparing the anthelmintic effects of ascaridole with those of the whole oil. It is our impression, however, that ascaridole is slightly more efficacious than the whole oil.) It would seem that Hall and Hamilton

TABLE 3

Trial treatments with 1.20 mls of ascaridole, followed ten days later by a test treatment of 2.00 mls of oil of chenopodium

CASE NUMBER	WORMS HARBORED	WORMS REMOVED BY TRIAL TREATMENT	WORMS REMOVED BY TEST TREATMENT	PERCENTAGE OF WORMS REMOVED BY TRIAL TREATMENT
1	37	37		100.00
2	172	166	6	96.51
3	4	2	2	50.00
4	9	9		100.00
5	40	40		100.00
6	10	10		100.00
7	27	25	2	92.57
8	8	6	2	75.00
9	303	300	3	99.99
10	273	270	3	98.90
11	57	57		100.00
12	42	38	4	90.47
13	20	20		100.00
Total.....	1,002	982	20	98.00

redistilled a part of the ascaridole, along with the terpenes, in their "lighter fraction," and that this fraction contained sufficient ascaridole to produce a fair anthelmintic effect. It is also probable, as Nelson suggests, that they allowed the oil to become overheated in the bath, thus causing decomposition and loss of part of the ascaridole as their heavier fraction was distilled over. Certainly the results obtained with the lighter fraction furnished us by Nelson do not warrant the statement that "the greatest anthelmintic efficiency resides in the lightest fraction of the drug," since practically no hookworms were expelled by the

lighter fraction which we used. On the other hand, 98 per cent of the worms harbored were expelled by the heavier (ascaridole) fraction when used in doses which corresponded to 60 per cent of the whole oil. That the heavier fraction is more toxic cannot be

TABLE 4

Symptoms produced by the terpenes (2.00 mls), ascaridole (1.20 mls) and whole oil of chenopodium (2.00 mls)

SYMPTOMS OBSERVED	TERPENES	ASCARIDOLE	WHOLE OIL
Burning sensation and flushing of face.....	5(±)	8(±), 10(±)	3(±), 10(±)
Dizziness.....	3(±), 5(±), 7(±), 11(±), 14(±), 15(±)	2(±), 4(-), 5(±), 6(±), 9(±), 11(±), 13(±)	1(±), 2(±), 4(±), 5(±), 6(±), 7(±), 8(±), 11(±)
Headache.....	5(±), 7(±), 8(±)	8(±), 10(±)	3(±), 14(±), 10(±)
Burning sensation in stomach.....	5(±)	6(+)	3(±)
Nausea.....	None	6(±)	3(±)
Vomiting (not produced by purgative).....	None	None	None
Burning sensation and tingling, palms, soles.....	None	1(+), 8(±), 10(±)	3(±), 10(±)
Numbness of extremities.....	None	1(+), 4(±)	10(±)
Tinnitus aurium....	None	8(±)	3(±)
Photopsia.....	None	None	None
No symptoms.....	1, 2, 4, 6, 9, 12, 13	3, 7	12, 16

The severity of symptoms are indicated as follows: (±) very light; (±) moderately severe; (+) severe. The numbers refer to case numbers in the foregoing tables, with the exception of the terpenes. No tabulation of the results of the second series of treatments with the terpene fraction is given in the text.

denied; but the toxic principles of the drug are responsible for its therapeutic effect, as in the case with practically all drugs. Hence it may be stated that no pharmacologic paradox has been established. It is undoubtedly true that the product marketed as oil of chenopodium should be redistilled, and that such a procedure would add to the safety and value of the drug. Not for

reasons set forth by Hall and Hamilton, however, since it does not seem possible to eliminate the fraction which has more irritant and toxic properties without eliminating the anthelmintic principle of the drug. Nelson (10) calls attention to the fact that ascaridole, the anthelmintic ingredient of the drug, is easily decomposed by heat and when the distillation is carried out with low pressure steam, without observing the necessary precautions as to temperature, the percentage of ascaridole will be low, the major part remaining in the distillation residue as decomposition products. Our experience with the drug has shown conclusively that the product marketed as "oil of chenopodium" varies greatly in anthelmintic potency. The real reason for redistilling the product would therefore seem to rest on the fact that it is entirely feasible to standardize the drug by fractional distillation. Nelson states that "no difficulty was encountered in obtaining ascaridole of constant boiling-point by fractionating oil of chenopodium under diminished pressure." If this be true, it would seem that a fraction of the drug which has a constant anthelmintic quotient could be easily separated from the whole oil, thus avoiding the uncertainty which one always has as to the anthelmintic potency of the drug on beginning the use of a new lot which has not been tested practically to determine its anthelmintic quotient.

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THE INFLUENCE OF THE ARTERIAL BLOOD SUPPLY TO THE LIVER ON HEMOGLOBIN CONCENTRA- TION IN CERTAIN ACUTE CONDITIONS¹

PART I

PAUL D. LAMSON, A. F. ABT, C. A. OOSTHUISEN AND S. M. ROSENTHAL

From the Pharmacological Laboratory of the Johns Hopkins University

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The problem of how the acute polycythemia produced by the intravenous injection of epinephrine takes place has been the subject of investigation in this laboratory for several years. It was considered that sufficient experimental proof had been obtained to show that this process takes place in the liver and Lamson and Roca (1) suggested the following as an explanation of how the increase in number of erythrocytes per unit volume of blood occurs. It was shown by Lamson that epinephrine although causing a marked polycythemia in the dog does not change the red cell content of the blood in the rabbit and that excitement also causes an increase in the number of red cells in the dog and cat, but has no such effect in the rabbit (2). At this same time Mautner and Pick (3) working on shock poisons found that epinephrine obstructs the flow through the perfused liver of the dog but causes no obstruction to flow in the rabbit's liver. Bainbridge and Trevan (4) following this work of Mautner and Pick found that there is a great increase in portal pressure after the injection of epinephrine but no change in the cava pressure in the dog. Finally Arey and Simonds (5) showed that the hepatic veins of the dog are heavily coated with muscle

¹ These hemoglobin concentration curves were given at the meetings of the American Society for Pharmacology and Experimental Therapeutics at New Haven, December 28 to 30, 1921 and a summary of the results will be found in the Proceedings, Journal of Pharmacology and Experimental Therapeutics, XIX, 1922, 267.

while those of the rabbit have a very small amount of musculature. From these data Lamson and Roca suggested that epinephrine causes a constriction of the hepatic veins, an obstruction to the flow of blood through the liver, an increased filtration pressure within the liver vessels, passage of fluid into the liver lymphatics causing a concentration of the blood and an increase in the number of erythrocytes per unit volume of blood. In order to test the validity of this theory and see if other substances act on the hepatic veins, the same method employed by Lamson and Roca was used, namely, the injection of large amounts of salt solution in a unit of time and the plotting of hemoglobin concentration curves. This method like all others dealing with blood concentration, and not the quantity of blood, can give only an index of the processes taking place but no quantitative proof of fluid exchange.

We have at the present time no method of determining blood volumes as has been pointed out by Lamson and Nagayama (6) and Lamson and Rosenthal (7). One of the chief difficulties is that we cannot tell whether the red cells are evenly distributed throughout the circulation normally or more especially in conditions of change. It is well known that there is a difference in blood concentration in the capillaries of the skin and in the deep vessels in such conditions as shock, Cannon (8), cold, Bostrum (9), and pernicious anemia, Duke (10), but very serious doubt is generally experienced by physiologists as to the ability of any one organ, as the liver, to actually store an appreciable amount of erythrocytes, and it is the prevailing custom to use hemoglobin concentration curves as indices of blood volume changes. It is hoped that the experiments here presented will demonstrate the possibility of red cell storage, and the error of using hemoglobin concentration curves alone as an index of blood volume change.

Method

This is described in detail by Lamson and Roca (1). It consists of the injection of 25 cc. of 0.8 per cent sodium chloride per kilo in exactly ten minutes in normal dogs but 20 per cent

less salt solution after removal of the liver. The drugs are mixed with this solution before injection, the object of using this large amount of salt solution is simply to exaggerate the hemoglobin changes after the injection of these drugs, thus making the method more accurate on account of greater variation in hemoglobin concentrations. In figure 1, a composite curve of 8 normal dogs is given which is taken as the standard

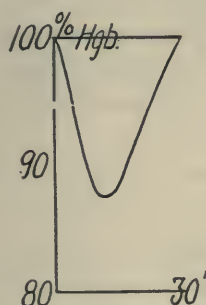


FIG. 1

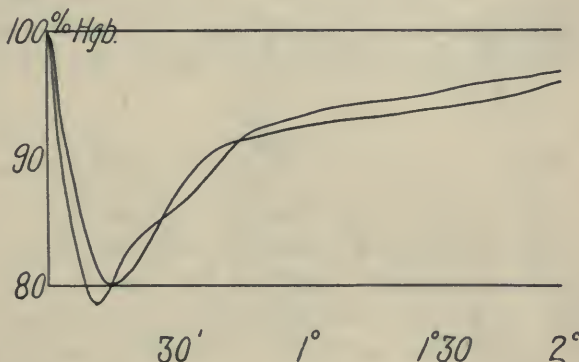


FIG. 2

FIG. 1. A COMPOSITE CURVE TAKEN FROM FIGURE 1, LAMSON AND ROCA (1), SHOWING THE HEMOGLOBIN CONCENTRATION CURVE AFTER THE INJECTION OF 25 CC. OF 0.8 PER CENT NaCl IN EXACTLY TEN MINUTES

This curve is taken as the standard—all other hemoglobin concentration curves being produced in the same way except for the addition of a drug to the solution injected.

FIG. 2. CURVES FROM LAMSON AND ROCA (1) SHOWING THE EFFECT OF REMOVAL OF THE LIVER FROM THE CIRCULATION

Twenty per cent *less* salt solution was injected to allow for the blood removed by shutting off the liver.

curve of hemoglobin concentration brought about by the injection of the salt solution alone. It will be noticed that there is a fall in hemoglobin concentration to about 85 per cent with fairly rapid return to normal in about thirty minutes. One is tempted to speak of such a curve as showing first a dilution of the blood and the rise as indicating the loss of fluid with a resulting concentration. One must however remember that

a fall in hemoglobin concentration might also be caused by a diminution of the number of corpuscles present in the circulation due either to cell destruction or storage of red cells out of active circulation, and the rise of hemoglobin concentration could equally well be due to the addition of red cells to the circulating blood.

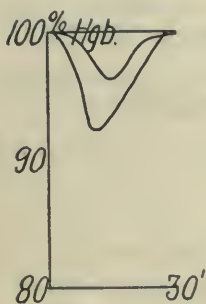


FIG. 3

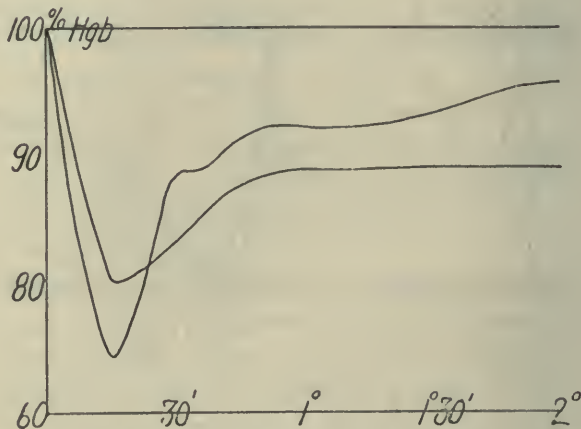


FIG. 4

FIG. 3. FROM LAMSON AND ROCA (1) SHOWING THE EFFECT OF THE ADDITION OF 0.9 MGM. OF EPINEPHRINE PER KILO TO THE SALT SOLUTION INJECTED

FIG. 4. CURVES SHOWING THE EFFECT OF THE ADDITION OF 0.9 MGM. OF EPINEPHRINE PER KILO TO THE SALT SOLUTION INJECTED (20 PER CENT LESS THAN THE STANDARD AMOUNT TO ALLOW FOR THE BLOOD REMOVED IN SHUTTING OFF THE LIVER)

It will be noticed that epinephrine has no appreciable effect after removal of the liver (compare with figure 2). From Lamson and Roca (1).

The effect of the removal of the liver

Figure 2 shows the hemoglobin concentration curve after removal of the liver from the circulation Lamson and Roca, (11). It will be noticed that there is a great prolongation of the depression in hemoglobin concentration.

The effect of epinephrine before and after removal of the liver

Figure 3 shows the effect of the addition of epinephrine to the salt solution injected and it will be noticed that there is a smaller depression in the hemoglobin curve with a more rapid return to normal. Figure 4 shows the effect of epinephrine on the hemoglobin concentration curve after the removal of the liver and it will be seen that this curve is practically the same as that in figure 2 in which no epinephrine was added, showing that epinephrine has little if any action outside the liver on hemoglobin concentration.

Histamine

Figure 5 shows the difference in hemoglobin curves produced by the injection of varying doses of histamine phosphate. Dale and Laidlaw (12) have shown an increased concentration of hemoglobin after the injection of histamine and one would expect a smaller depression of the curve and a more rapid return to normal but it is interesting that in spite of the large amount of fluid injected no fall in hemoglobin concentration occurs and that even a very marked concentration of blood may take place.

Histamine after removal of the liver

Figure 6 gives two curves of hemoglobin concentration, after the injection of histamine, one of which was taken after the removal of the liver from the circulation by the ligation of the arterial blood supply to the liver and anastomosis of the portal vein and vena cava, the second curve after the liver was completely extirpated. It will be seen that this curve is not like that of figure 2, the curve of normal salt after removal of the liver, but that the hemoglobin concentration returns to normal very rapidly. This appears to show that histamine unlike epinephrine, acts on other parts of the body rather than in the liver alone, and it would be impossible to explain the concentration of the blood occurring after this injection by a simple constriction of the hepatic veins only.

Barium chloride

Figure 7 shows the effect of the addition of large amounts of barium chloride to the salt solution injected. The curves obtained are very similar to those of epinephrine.

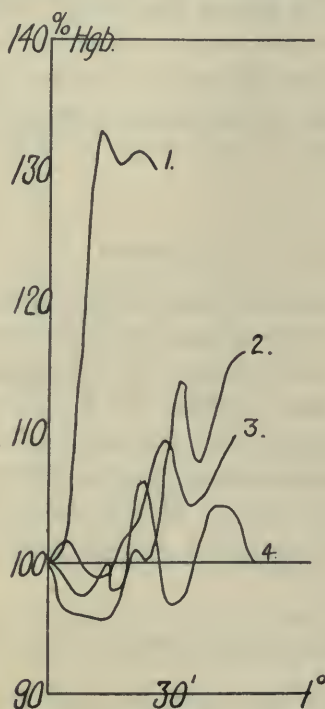


FIG. 5

FIG. 5. CURVES 1, 2, 3 AND 4 SHOW THE EFFECT OF THE ADDITION OF 3.0, 0.1, 0.01, AND 0.03 MGM. OF HISTAMINE PHOSPHATE PER KILO, RESPECTIVELY, TO THE SALT SOLUTION INJECTED

Nitrites

Hemoglobin concentration curves were plotted after the injection of sodium nitrite, nitroglycerin and the inhalation of amyl nitrite. Not enough experiments were done to obtain a satisfactory dose to give a good curve in all cases, some causing death, others not being large enough to be of any effect. The general shape of the curves obtained, fell between the normal salt curve and that of pituitrin.

Pituitrin

Small doses of pituitrin (0.15 cc. per kilo of Armour's pituitary liquid) have little if any effect on the hemoglobin curves (fig. 8). Very much larger doses (2 cc. per kilo) caused a marked depression of the curve with a very slow return to normal (fig. 9). This curve is very similar to that of figure 2 obtained after removal of the liver from the circulation.

Pituitrin and epinephrine

As pituitrin causes a prolongation of the hemoglobin depression while epinephrine hastens a return of the curve to normal these two drugs were mixed in maximum doses and injected in salt solution. It will be seen from figure 10 that the pituitrin action completely overcomes the action of the epinephrine.



FIG. 6

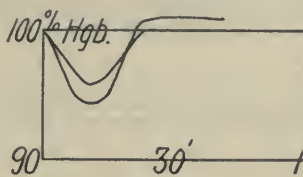


FIG. 7

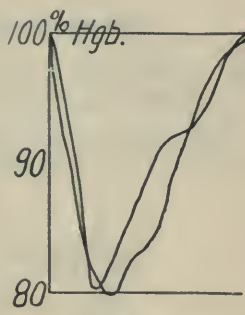


FIG. 8

FIG. 6. TWO CURVES SHOWING THE EFFECT OF 1.8 MCM. PER KILO OF HISTAMINE PHOSPHATE AFTER REMOVAL OF THE LIVER

In the first the liver has been excluded from the circulation by the ligation of the arterial blood supply to the liver, anastomosis of the portal vein and vena cava, and ligation of the portal vein at the hilus of the liver. In the second curve the liver was completely extirpated. It will be noted that unlike epinephrin, histamine has a definite action on hemoglobin concentration after removal of the liver.

FIG. 7. CURVES SHOWING THE EFFECT OF THE ADDITION OF 0.7 CC. OF A 1 PER CENT SOLUTION OF BARIUM CHLORIDE PER KILO TO THE SALT SOLUTION INJECTED

FIG. 8. CURVES SHOWING THE EFFECT OF 0.15 CC. PER KILO OF ARMOUR'S PITUITARY LIQUID TO TWO DOGS IN THE CUSTOMARY AMOUNT OF SALT SOLUTION

The curves are very similar to the standard salt curves.

Pituitrin and histamine

Here again pituitrin when mixed with the maximum doses of histamine completely overcomes the histamine action (fig. 11).

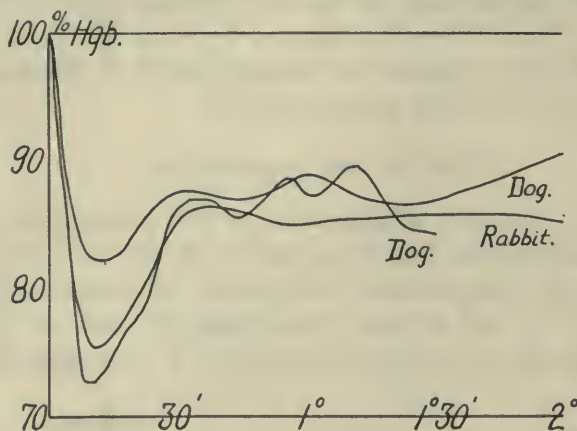


FIG. 9. CURVES OBTAINED AFTER THE INJECTION OF 2 CC. PER KILO OF ARMOUR'S PITUITARY LIQUID WITH THE USUAL AMOUNT OF SALT SOLUTION

The prolonged depression of hemoglobin is very marked and gives a curve similar to that of the removal of the liver (fig. 2).

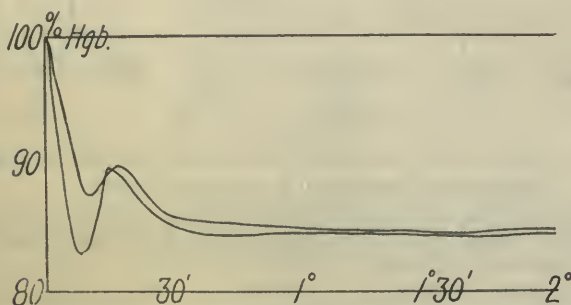


FIG. 10. CURVES TAKEN AFTER INJECTION OF EPINEPHRINE (0.9 MGM. PER KILO) AND ARMOUR'S PITUITARY LIQUID (2 CC. PER KILO) WITH THE USUAL AMOUNT OF SALT SOLUTION

It will be noticed that the pituitrin entirely overcomes the action of epinephrine shown in figure 3.

The effect of epinephrine, histamine, barium chloride, nitrites, and pituitrin alone, on the hemoglobin concentration

Epinephrine (13), histamine (Dale and Laidlaw, 14), barium chloride (Underhill, 15) when given alone all cause a concentration of the blood. Nitrites (Underhill, 16) and pituitrin cause no concentration. See figure 12.

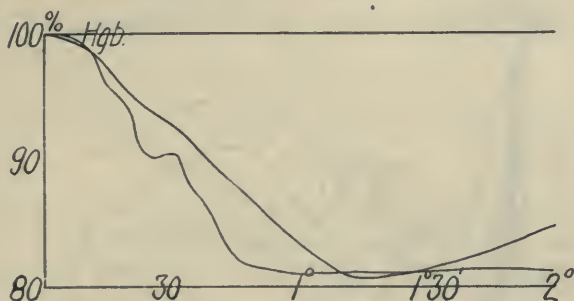


FIG. 11. CURVES SHOWING THE EFFECT OF HISTAMINE PHOSPHATE, 1.8 MGM. PER KILO AND ARMOUR'S PITUITARY LIQUID, 2CC. PER KILO IN THE HEMOGLOBIN CONCENTRATION CURVE WHEN INJECTED IN THE STANDARD AMOUNT OF SALT SOLUTION

It will be seen that here again the pituitrin completely overcomes the histamine effect.

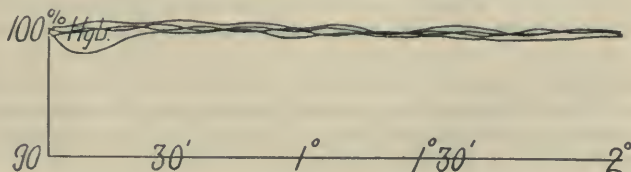


FIG. 12. FOUR CURVES OF HEMOGLOBIN CONCENTRATION AFTER THE INJECTION OF 2 CC. OF ARMOUR'S PITUITARY LIQUID ALONE

Plasma protein concentration curves

Theoretically these curves should be of very little value in determining the loss or retention of fluid as the liver capillaries (Starling, 17) if no others, are capable of allowing the passage of plasma proteins as well as fluid through their walls. In some cases plasma only may leave the vessels, while in others both may pass freely, and in still others the two may leave the

circulation in various ratios. It is then evident that any one curve may be a composite of all these factors. The following curves (fig. 13) are given to show the actual conditions taking place. It is evident from the same general shape of these curves, and the great differences in the hemoglobin curves, that both types cannot be taken as true indices of fluid exchange.

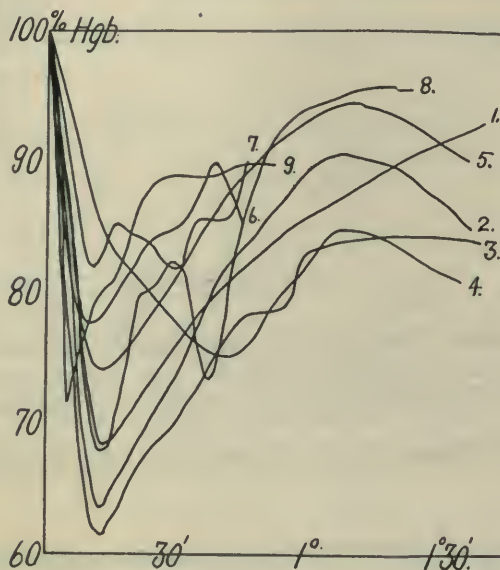


FIG. 13. CURVES 1, 2, AND 3 ARE PLOTTED FROM PLASMA PROTEIN CONCENTRATIONS AFTER THE INJECTION OF 2 CC. PER KILO OF ARMOUR'S PITUITARY LIQUID IN 25 CC. OF SALT SOLUTION PER KILO. CURVES 4 AND 5 SHOW THE CHANGES IN CONCENTRATION OF ANOTHER SUBSTANCE, VITAL RED INJECTED TWENTY-FOUR HOURS PREVIOUSLY AFTER THE INJECTION OF PITUITRIN. CURVE 6 IS THE PLASMA PROTEIN CURVE AFTER INJECTION OF SALINE ALONE. CURVE 9 IS THE EPINEPHRINE AND SALINE CURVE AND CURVES 7 AND 8 ARE HISTAMINE AND SALINE CURVES

It will be seen that all of these curves are of the same general shape.

Vital red concentration curves

As plasma proteins are normal to the blood and could be secreted from the tissues into the blood stream and thus upset the curve, another substance foreign to the blood, with a large molecule, was injected intravenously, and allowed to mix until

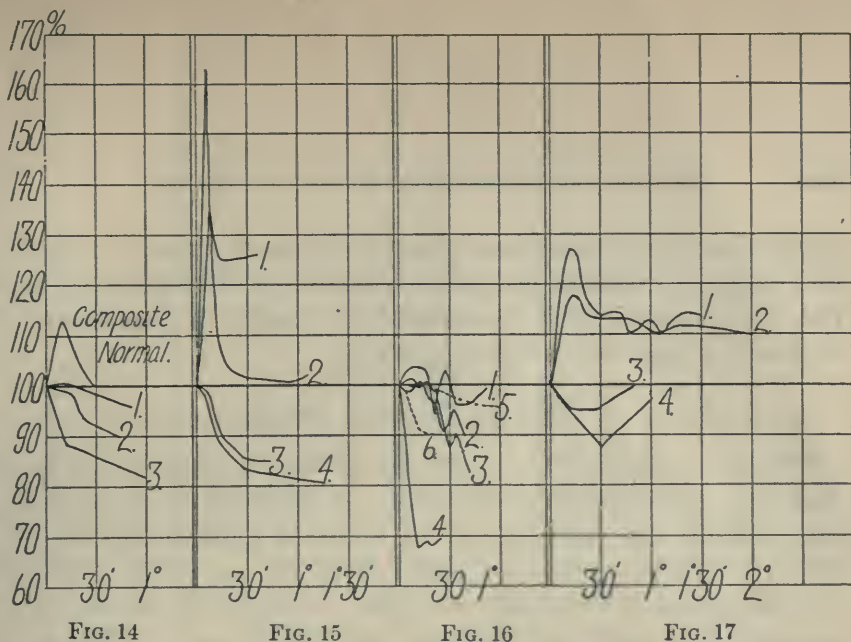


FIG. 14. SERIES OF CURVES TO COMPARE A CURVE OF VOLUME CHANGE REPRESENTED BY PLASMA VOLUME DETERMINATIONS DONE BY THE KEITH, ROWNTREE AND GERAGHTY METHOD, AND VOLUME CHANGE AS INDICATED BY PLOTTING HEMOGLOBIN CONCENTRATION

All of these experiments were carried out on dogs under ether anesthesia, and blood samples taken from the jugular vein. The hemoglobin concentration was determined by drawing about 0.5 cc. of blood with a small needle and syringe, measuring an exact amount of this (0.4 cc.) and diluting in 100 cc. of dilute HCl. After standing one hour the solutions were read in the Dubosq colorimeter using the first as the standard.

The upper curve is a composite of hemoglobin concentrations after the injection of 25 cc. of 0.8 per cent saline per kilo intravenously in ten minutes, in 8 dogs. (See Lamson and Roca, Jour. Pharm. Exper. Therap., 1921, xvii, 483.) The curves are the inverse of the actual concentrations to express blood volume change. The lower three curves are volumes determined by the dye method at intervals after the injection of the same amount of salt. It will be seen that although there is uniformity of each method *the two methods give exactly opposite results.*

FIG. 15. IN THIS EXPERIMENT 5 GRAMS GLUCOSE PER KILO WERE INTRAVENOUSLY INJECTED IN A CONCENTRATED SOLUTION, AND THE VOLUME CURVES PLOTTED AS BEFORE BY THE DYE AND HEMOGLOBIN METHODS

It will be seen that there is an enormous increase of volume by the hemoglobin method but a decrease by the vital red method. Here again *the two methods give opposite results.*

FIG. 16. SHOWS SIX CURVES ALL OF WHICH SHOW A DOWNWARD TREND REPRESENTING A DECREASE IN BLOOD VOLUME

These curves were obtained by the plasma volume method in curves 5 and 6, and the hemoglobin method in 1, 2, 3, and 4. In this experiment varying amounts of histamine phosphate were added to the salt solution injected, and in this condition the two methods *agree.*

FIG. 17. THESE CURVES WERE OBTAINED BY THE DYE AND HEMOGLOBIN METHODS AFTER THE ADDITION OF LARGE AMOUNTS OF ARMOUR'S PITUITRIN TO THE SALT SOLUTION INJECTED

Curves 1 and 2 were plotted by the hemoglobin method and 3 and 4 by the dye method. It will be seen here again that *the two methods give opposite results.*

equilibrium had been established which took place in twenty-four hours. Pituitrin plus salt solution was then injected and the concentration of vital red plotted. The curve thus obtained is shown in figure 13, curves 4 and 5.

Vital red "volume" curves

"Plasma volumes," were determined by the method of Keith, Rowntree and Geraghty (18) after the injection of epinephrine, salt solution and pituitrin by the vital red method (figs. 14, 15, 16, 17). These curves are taken from the paper by Lamson and Rosenthal (7) and the change in "volume" by this method is shown in no way to correspond with the change in volume of blood plotted from hemoglobin concentrations. A discussion of the causes of these differences is given in that paper. The curves are here repeated for the sake of completeness and as an aid to determining what changes actually took place.

Summary

We have seen that epinephrine, histamine, barium chloride, nitrites and pituitrin have definite but different actions on blood concentration when injected alone or in 25 cc. of 0.8 per cent sodium chloride per kilo. Epinephrine, histamine and barium chloride decreasing the dilution of the blood with injections of salt solution and increasing the rate of return of hemoglobin to normal. Nitrites to some extent and pituitrin very definitely cause the opposite, a marked fall and a very slow return to normal of the hemoglobin curves. Besides the difference in action of these substances we are interested in the cause of these changes; whether there has been fluid loss or retention alone, or whether there has been a redistribution of red cells also. These experiments were undertaken to analyze the function, if any, of the hepatic veins in causing obstruction to blood flow and filtration of fluid into the liver tissue. On this account the changes and cause of change in venous pressures with these different drugs were taken up.

PART II. THE RELATION BETWEEN ARTERIAL, VENOUS (PORTAL
AND CAVA) PULMONARY PRESSURES AND LYMPH FLOW
AND THE HEMOGLOBIN CONCENTRATION CURVE

Carotid pressures

Carotid pressure curves have been taken in all of these conditions during the injection of salt solution until the pressure has returned to normal. It is found that there is no relation between the arterial pressure curve and the hemoglobin concentration curve. For example, the arterial pressure is high with epinephrine, barium chloride and pituitrin. The hemoglobin curve falls less and returns to normal more quickly with epinephrine and barium chloride but the depression is more marked and prolonged with pituitrin. Histamine causes a fall in pressure and a concentration of the hemoglobin, while the nitrites cause a fall in pressure but a prolonged fall in the hemoglobin curves. Finally nitrites produce a prolonged fall in the hemoglobin curve similar but not as marked as that with pituitrin but the arterial pressure is high with the pituitrin, and low with the nitrites.

Pulmonary artery and venous pressures

The portal pressure was measured by thrusting a needle through a branch of this vein and tying it in, leaving the end of the needle in the portal vein and connecting with a 3 per cent citrate manometer. The cava pressure was taken from a cannula inserted into one femoral vein. The pressure in the pulmonary artery was taken from a cannula tied into one branch of the pulmonary artery, reached by an incision between two ribs, artificial respiration being used. A wash-bottle was attached to the manometer and every little while 1 cc. or so of citrate allowed to flow through the cannula, in this way checking the curve. In all cases the drug was added to and injected in the regular dose of 25 cc. of salt solution per kilo. Venous pressures have been taken by different observers before, but the pressures here recorded were taken using large amounts of sodium chloride solution and are of interest only in

relation to the hemoglobin concentration curves in these special cases.

It will noticed from the curves (figs. 18 and 19) given that pituitrin and the nitrites (figs. 20 and 21) cause practically no increase, but a fall in both cava and portal pressures, and that the hemoglobin concentration curves with these two sub-

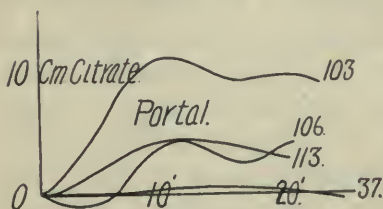


FIG. 18

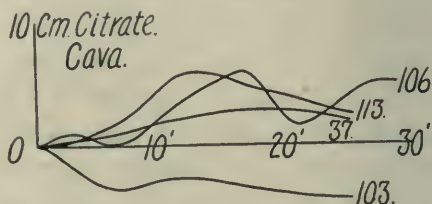


FIG. 19

FIG. 18 AND 19. PORTAL AND CAVA PRESSURES AFTER THE INJECTION OF 2 CC. PER KILO OF ARMOUR'S PITUITARY LIQUID IN 25 CC. OF 0.8 PER CENT NaCl SOLUTION PER KILO

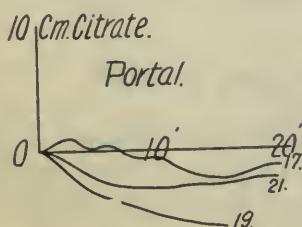


FIG. 20

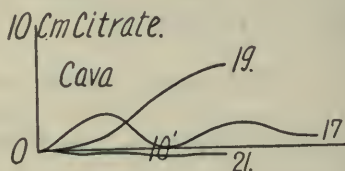


FIG. 21

FIG. 20 AND 21. PORTAL AND CAVA PRESSURES WITH 5 DROPS SPIRITS OF NITROGLYCERIN PER KILO (19 TO 21) AND 10 MGM. PER KILO OF SODIUM NITRITE IN 25 CC. OF SALT SOLUTION PER KILO (17)

stances show no concentration of the blood. Histamine (figs. 22 and 23) causes usually a moderate rise in both portal vein and vena cava, but the portal is more pronounced. Epinephrine has been shown to cause a marked increase in portal pressure but only a slight change in cava pressure (Bainbridge and Trevan, 19). This has been confirmed by us. Ba-

rium chloride causes an increase in venous pressure (figs. 24 and 25) and a concentration in the hemoglobin curve. The actual increase in venous pressure is almost always more marked in the portal vein than the vena cava. Barium chloride causes enormous periodical variations in portal pressure due possibly to peristalsis which is very pronounced after the injection of

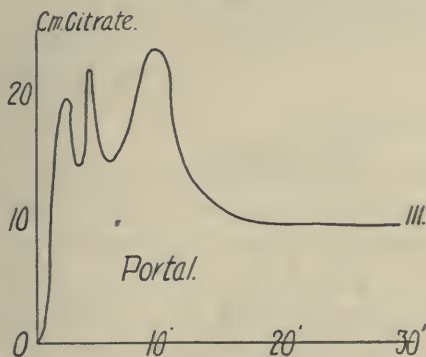


FIG. 22

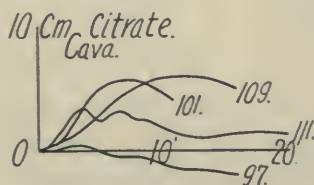


FIG. 23

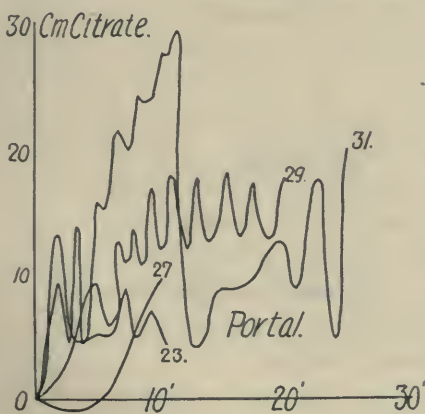


FIG. 24

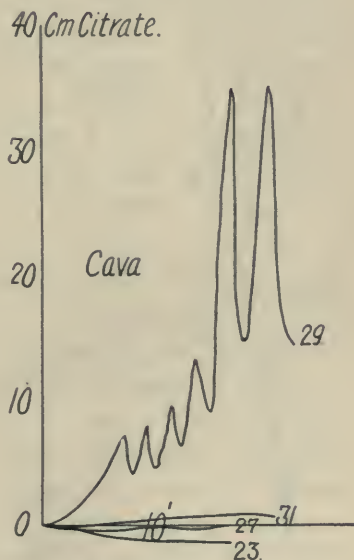


FIG. 25

FIGS. 22 AND 23. CURVES SHOWING THE EFFECT ON VENOUS PRESSURE OF 1.8 MGm. OF HISTAMINE PHOSPHATE IN 25 CC. OF 0.8 PER CENT NaCl PER KILO

FIGS. 24 AND 25. VENOUS PRESSURES WITH 0.7, 1.0, 0.8 AND 0.35 CC. OF 2 PER CENT NBAaCl₂ PER KILO IN CURVES 23, 27, 29 AND 31 RESPECTIVELY IN 25 CC. OF 0.8 PER CENT NaCl PER KILO

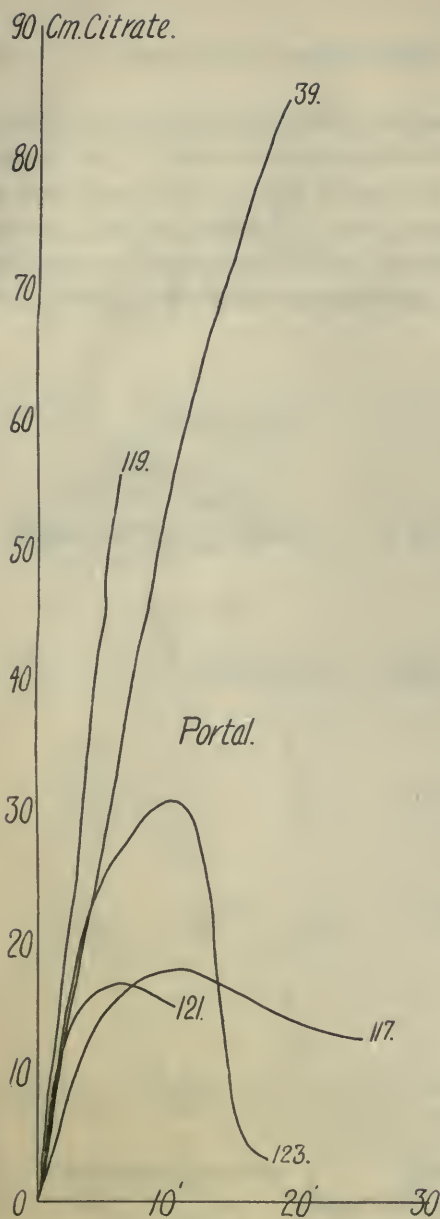


FIG. 26

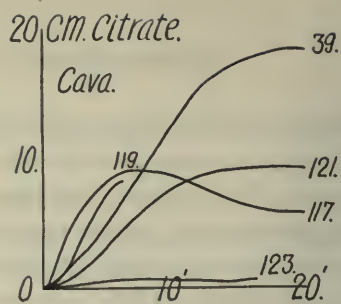


FIG. 27

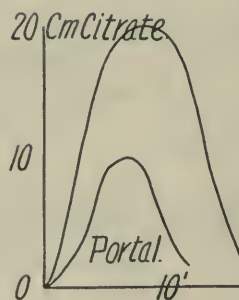


FIG. 28

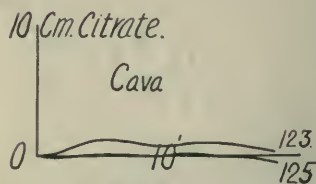


FIG. 29

FIGS. 26 AND 27. VENOUS PRESSURES WITH 0.9 MGM. OF EPINEPHRIN PER KILO AND 2 CC. OF ARMOUR'S PITUITARY LIQUID PER KILO IN 25 CC. OF 0.8 PER CENT NaCl PER KILO

FIGS. 28 AND 29. VENOUS PRESSURE CURVE WITH A MIXTURE OF 1.8 MGM. OF HISTAMINE PHOSPHATE AND 2 CC. OF ARMOUR'S PITUITARY LIQUID IN 25 CC. OF 0.8 PER CENT NaCl PER KILO

barium chloride. There appears then to be a relationship between increased venous pressure and hemoglobin concentration. This however is not so in all cases for if epinephrine and pituitrin are mixed and injected together there is an enormous increase in portal pressure (figs. 26 and 27), in one case the citrate solution rising over a 90 cm. manometer, but there was no concentration in the hemoglobin curve. The same thing occurs to a lesser extent with a mixture of histamine and pituitrin (fig. 28 and 29).

Pulmonary pressures

Pulmonary pressures were obtained by opening the chest by an incision between two ribs on the left side and cannulating a branch of the pulmonary artery. The changes in pressure were sometimes recorded with a mercury manometer but they were so slight that this was not practical and a citrate manometer was used, the changes being read and noted but not recorded graphically. Drinker's method (20) of recording pressures with a light membrane manometer was not known at that time. It was found that epinephrine, histamine, barium chloride, nitrites or pituitrin never cause an increase in pulmonary pressure which is in the least proportional to the increase in pressure found in the portal vein or vena cava, in the dog. From the observations of Dale and Laidlaw (21) and Abe (22) the action of histamine on the pulmonary artery of the cat appears to be more pronounced. From these experiments one is justified in concluding that the increase of vena cava pressures found are not directly due to an increase in the pressure in the pulmonary artery. Furthermore it was shown that after the pulmonary veins are partly or totally occluded by gently pressing on them with the fingers *there is no rise in the cava or portal pressures regardless of an increase of as much as 40 cm. citrate in the pressure in the pulmonary artery.* We are not aware that this fact has been observed before, many believing that the increase in pressure in the pulmonary artery will cause a back pressure in the vena cava. The arterial pressure as is well known falls in direct relation to the obstruction of the pulmonary vein. The vena

cava pressure is then not due to any direct increase in pressure in the pulmonary artery, brought about by the injection of these drugs.

Bronchi

The effect of large doses of epinephrine, histamine, barium chloride, nitrites and pituitrin have been studied on the bronchi (on account of possible obstruction of blood flow through the lungs) and it will be seen that there is no relation between bronchial constriction and venous pressure. (The curves are not reproduced here for lack of space.) Histamine causes very intense bronchial constriction and marked increase in venous pressure. Pituitrin causes the next most intense bronchial constriction but the action is slight in comparison with histamine and causes if anything a fall in venous pressure. Epinephrine causes a relaxation of the bronchi and a rise of venous pressure. Barium chloride causes if anything a dilatation of the bronchi and rise of venous pressure. Bronchial constriction cannot then be said to be the cause of increased venous pressure in these conditions.

Lymph flow

The lymph flow from the thoracic duct was studied after the injection of salt solution and all of the above mentioned drugs by cannulating the thoracic duct. After the injection of salt solution there is a marked increase in the flow of lymph and after the injection of the salt solution with these drugs there is also an increased flow of lymph in some cases very marked, but the lymph flow (10 to 15 cc.) during the time of marked changes in the hemoglobin concentration curve is entirely out of proportion to the fluid exchange necessary to cause the change in hemoglobin concentration which has taken place. If then fluid is lost into the liver it must be retained to a large extent in the liver capillaries. The great swelling of the liver observed in these dogs would agree with this.

Summary

It will be seen that there is no relation between the hemoglobin concentration curves and the arterial pressure curves. This might be expected as capillary pressure curves do not run parallel with arterial pressures in all cases and one would not expect filtration of fluid through the thick arterial wall. It will however have been noticed that with the drugs causing an increase in venous pressure, epinephrine, histamine, barium chloride, that there is an increased rate of hemoglobin concentration while the nitrites and pituitrin neither of which causes an increase in venous pressure, cause no increased rate of hemoglobin concentration. This might be expected for any increase in venous pressure necessitates an increase in capillary pressure and in this way mechanically increases the tendency to filter fluid through the capillary walls. There are however exceptions to this observation.

In the cases where histamine and pituitrin, and also epinephrine and pituitrin, are mixed and injected together, there is an exception to the relationship between venous pressures and hemoglobin curves. Here in spite of the highest venous pressures recorded at any time, there is a very prolonged fall of the hemoglobin curve. Considering for a moment that a fall in the hemoglobin curve might be due to fluid loss one might expect that an increase in venous pressure would cause a loss of fluid through the thin walled capillaries in which there must be as great a pressure as in the veins. We have seen that there is this apparent loss of fluid when there is high venous pressure except in these two cases where pituitrin is given together with another drug. As pituitrin causes a prolonged fall in the hemoglobin curve, one might not expect fluid loss with this drug alone especially as there is no increase of venous pressure after its injection, but it is hard to conceive of an increase of pressure of 100 cm. of citrate without a filtration of fluid through the capillaries. A year or so after this observation was made Krogh (23) announced his discovery that pituitrin constricts the capillaries in the frog. It seemed for a time that in the cases here

described with this high venous pressure either a change in the permeability of the vessels or a constriction of the capillaries reducing the filtration surface, were the chief factors to be considered, but the possibility of a redistribution of red cells had to be taken into consideration. There might be either a loss of fluid from the circulation or a storage of red cells. Considerable light was thrown on the possible action of pituitrin by the following experiment with ligation of the hepatic artery.

PART III. THE EFFECT OF THE EXCLUSION OF THE ARTERIAL BLOOD SUPPLY TO THE LIVER ON THE HEMOGLOBIN CONCENTRATION

Experiments were under way to test out the actions of pituitrin on the capillaries, and vessel permeability in dogs, when the following results from ligation of the arterial blood supply to the liver were obtained which show at least one method by which pituitrin could cause the changes found in hemoglobin concentrations, but which do not prove that pituitrin acts in this way only. It was previously found by one of us (24, 25) that the hepatic artery may be clamped and unclamped at intervals of one-half hour with no effect on the red corpuscle content of the blood but if the arterial blood supply to the liver is shut off and then epinephrine injected no increase in the red cell content of the blood takes place, as always occurs in the normal dog. But if one-half hour or so later the clamp is removed from the hepatic artery, thus restoring the arterial circulation to the liver, there is an immediate increase in the number of erythrocytes per unit volume of blood. If the theory of Lamson and Roca is correct that epinephrine causes obstruction to blood flow to the liver by a constriction of the hepatic veins and the filtration of fluid into the liver lymphatics due to the high portal pressure it is evident that if fluid is lost into the liver tissue, the number of red cells per unit volume of blood in the liver capillaries is increased in proportion to the amount of fluid lost. Apparently with the normal circulation this concentrated blood is carried out of the liver and the red cells distributed throughout the circulation. If, however, the driving force of the hepatic

artery circulation is removed, the red cells concentrated in the liver capillaries by the loss of fluid into the liver lymphatics may be sedimented in this organ on account of the very sluggish blood flow shown by the high portal pressure. That some such process does occur seems likely from the fact that after the injection of epinephrin with the hepatic artery tied, there is the usual increase in portal pressure, swelling of the liver and increased lymph flow and that after the removal of the ligature when the effect of epinephrine has worn off the red cells appear in the general circulation as evidenced by an increase in their number per unit volume of blood.

As we have previously shown, a fall in hemoglobin concentration even during the injection of large amounts of fluid might be due either to dilution of the blood by the fluid injected, or to a storage of red cells. If shutting off the arterial blood supply to the liver could cause a storage of red cells in the liver as was thought to be the case with epinephrine, the effect of pituitrin might be merely a shutting down of the arterial blood supply to the liver causing a prolonged dilution of the blood due to storage of red cells rather than to the absence of fluid loss. In order to test this hypothesis the arterial blood supply to the liver was entirely shut off by cutting and ligating all tissue going to the liver except the portal vein, and the tissues about the exit of the hepatic veins. If the hepatic artery is thus ligated and these different drugs injected, it will be seen that in spite of the variety of curves obtained in the normal dog with salt solution, epinephrine and histamine, that these all fall into one general type of curve when the hepatic artery is ligated Fig. 30. Does such a change in the shape of the curves mean fluid retention or storage of erythrocytes?

It is of interest to note in this complex mechanism that after removal of the liver from the circulation and the injection of histamine, the hemoglobin curve (fig. 6) is quite similar to the normal salt curve, and as above stated this shows in contrast to epinephrine that histamine acts after removal of the liver. If, however, the histamine curve after ligation of the hepatic artery is studied it will be seen that there is a greater depression

of hemoglobin concentration than after entire removal of the liver; and that instead of a rapid return of hemoglobin to normal, the depression of the curve is of long duration. This would appear to show that ligation of the hepatic artery—a small part of the liver, has a greater effect, than the entire liver,

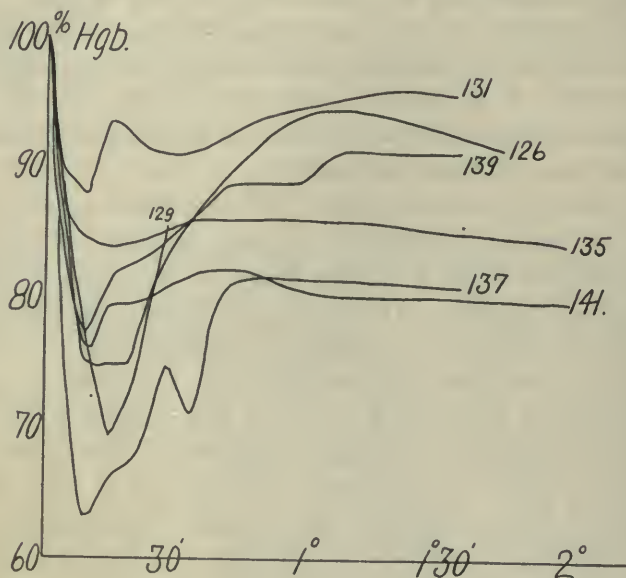


FIG. 30. CURVES SHOWING THE EFFECT OF SHUTTING OFF THE ARTERIAL BLOOD SUPPLY TO THE LIVER, ON THE HEMOGLOBIN CONCENTRATION CURVES AFTER THE INJECTION OF 25 CC. PER KILO OF 0.8 PER CENT NaCl WITH THE ADDITION OF EPINEPHRIN 0.9 MGM. PER KILO, CURVES 126, 129 AND 137 HISTAMINE 1.8 MGM. PER KILO, CURVES 131, 135, AND SALINE ALONE IN CURVES 139 AND 141

It will be noticed that all these curves are of the same general type—a marked fall in hemoglobin concentration and a very slow return to normal. A comparison of these hemoglobin curves with those in the normal dog will show the marked influence of ligation of the hepatic artery and the resemblance of these curves to those obtained with pituitrin, will also be seen.

as shown by its removal. If, however, the liver is thought of as a filter in which erythrocytes can be caught, thus reducing the hemoglobin concentration in the general circulation it is evident that this filtration action might have a greater effect on the curve than complete removal of the liver. This may be taken

as another bit of evidence in favor of a storage of erythrocytes in the liver after ligation of the hepatic artery and injection of drugs.

Discussion

The question naturally arises as to what all these curves mean. They are of interest in the first place in showing definite differences in action of these drugs and give a method of distinguishing them. They are also of interest in showing the controlling influence of the circulation in the hepatic artery, but until adequate quantitative methods for blood volume studies

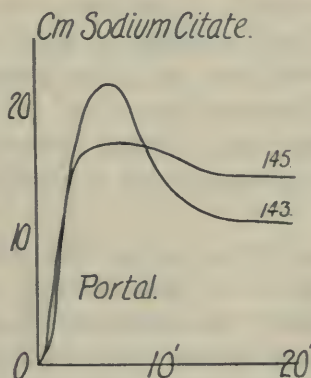


FIG. 31. CURVE SHOWING THE PORTAL PRESSURE IN 2 DOGS AFTER THE INJECTION OF 0.9 MG. OF EPINEPHRINE PER KILO AFTER LIGATION OF THE HEPATIC ARTERY

It will be noticed that in spite of the absence of concentration in hemoglobin curves with epinephrine when the hepatic artery is ligated there is the usual increase in portal pressure.

have been developed one cannot determine how these substances act. Pituitrin, for example, alone causes no increase in hemoglobin concentration, it also causes no decrease in hemoglobin concentration but added to the salt solution and injected it has a definite action; it *decreases* the rate of return of hemoglobin concentration to normal. If this should mean that pituitrin causes a retention of saline injected it would be an ideal therapeutic agent as it also raises the blood pressure. Besides this,

it completely overcomes the action of epinephrine and histamine both of which substances are thought to be increased in shock. But does pituitrin really cause fluid retention? One might believe that by capillary constriction, filtration would be lessened, but one obtains the same curve with simple ligation of the hepatic artery. Here with epinephrine for instance, there is a great increase of portal pressure (fig. 30) but, in spite of this, a curve suggestive of fluid retention and here there is no reason to believe a general capillary constriction but only absence of circulation in the hepatic artery. This question of the cause of changes in hemoglobin concentration is at present incapable of solution. A change in hemoglobin concentration is the resultant of four factors; addition of fluid or corpuscles to the circulating blood, or removal of fluid or corpuscles from the circulation. No one of these factors can be determined quantitatively and therefore the problem is incapable of solution. One can only collect as many data as possible and reason from them.

From the experiments here given and those previously published we feel that the liver undoubtedly plays an important part in these hemoglobin concentration changes in spite of the criticism of other investigators. We cannot agree with Scott (26) who believes the liver has nothing to do with acute polycythemia, but that the hemoglobin concentration varies directly with *arterial* pressure, or with Bostrum (27) who was unable to produce polycythemia with epinephrine in the first place, and explained away the part played by the liver found by Lamson, as due to a general cooling of the body from removal of the liver, thus upsetting the customary changes in hydrogen-ion concentration of the blood brought about by epinephrine, and the change of charge on the corpuscles which according to him is the cause of the observed increases in numbers of erythrocytes. We feel that on account of the very numerous investigators who have found a polycythemia after the injection of epinephrine that this point does not need further confirmation. Neither can we agree with Edmunds and Stone (28) who believe that the hemoglobin changes in acute epinephrine polycythemia are not

due to the liver but occur in the bone marrow, for which idea we find no proof, and against which in previous papers we have considerable data. Nor can we believe that these investigators could fail to obtain results if they had succeeded in shutting off the entire arterial blood supply to the liver as emphasized by Lamson (29). Another remarkable statement is made in an editorial in the Journal of the American Medical Association (30) in which Scott was referred to as having shown that fluid loss due to increased arterial pressure was the cause of epinephrine polycythemia. In this editorial one reads "Lamson believes that the number of corpuscles is altered by a splitting of old corpuscles, or a production of new ones, as well as storage in the liver." We must take this opportunity to disclaim such a statement. Bogert, Underhill and Mendel (31) also quote Lamson as having stated that excitement, etc., cause changes in hemoglobin concentration, an observation which they could not confirm. We feel that they would have been more successful if they had used *dogs or cats* instead of *rabbits* in which latter animals it had already been pointed out by Lamson (32) that it was impossible to produce an increase in the number of erythrocytes per unit volume of blood by either epinephrine or emotional stimuli.

Finally these experiments with pituitrin and especially with the mixtures of pituitrin and epinephrine and pituitrin and histamine, and also ligation of the hepatic artery although rather radical procedures, show the importance of red cell distribution emphasized by Lamson and Nagayama (6) in any blood volume or blood concentration studies, a statement criticized by Erlanger (33) and others as of chiefly theoretical interest. A consideration of the various factors involved with the artificial stimulus, intravenous injection of epinephrine, and the physiological stimulus, fright, in the hemoglobin concentration changes in dogs and rabbits, will demonstrate the necessity of consideration of red cell distribution in any physiological or pathological process and the impossibility of its solution on account of our utter inability at the present time to fix any of the four factors involved; namely, the amount of fluid or red cells added or lost from the circulation.

Conclusions

1. On account of our inability to determine quantitatively any of the four factors involved in blood concentration studies; namely, the amount of fluid or erythrocytes lost or added to the circulation, it is impossible to interpret the results here obtained in terms of fluid exchange between the blood and tissues.

2. Differences in effect on hemoglobin concentration have been shown between epinephrine, histamine, barium chloride, nitrites and pituitrin given in large doses.

3. Histamine acts in a large degree on the liver but on other organs as well, in contrast to epinephrine which appears to act on the liver only, as regards hemoglobin concentration.

4. Exclusion of the arterial blood supply to the liver has a very marked effect on the action of salt solution, and epinephrin and histamine, given in large amounts of salt solution, on hemoglobin concentration, reducing their widely divergent curves to one common type.

5. The action of pituitrin in overcoming the effect of histamine and epinephrine on blood concentration might be explained by a constrictor action on the hepatic artery; but this is unproven as yet.

6. It has been shown that there is no relation between arterial pressure and the hemoglobin concentration curves, but that with epinephrine, histamine and barium chloride all of which cause an increase in venous pressure, an increase in hemoglobin percentage takes place, while with nitrites and pituitrin, where there is no increase in venous pressure there is no increase in hemoglobin concentration. On the other hand high venous pressure occurs with a mixture of pituitrin, and epinephrine, and of pituitrin and histamine, with no increase in hemoglobin concentration, an action very similar to ligation of the hepatic artery. A variation in the arterial blood supply to the liver may thus obscure the action taking place after any of these drugs.

7. No relationship between bronchiole action, or changes in pressure in the pulmonary artery, and venous pressure could be made out.

8. It is suggested, but not proven, that exclusion of the arterial blood supply to the liver does not stop the filtration of fluid into the liver tissues if such a process takes place after the injection of a drug in the normal animal but that the absence of an increase of hemoglobin concentration in the great vessels is due to the retention of red blood corpuscles in the liver, the arterial blood supply to this organ being necessary to carry them into the general circulation from the place of hemoglobin concentration in the liver brought about by loss of fluid into the liver lymphatics.

9. The effect of drugs on the hepatic artery of different animals in relation to hemoglobin concentration curves is now being investigated.

10. These experiments add new evidence to the belief that the liver is the seat of the processes causing the acute polycythemia produced by the injection of epinephrine. They demonstrate that if a drug should sufficiently constrict the hepatic artery, fluid loss might occur with no change in hemoglobin concentration. Such a condition might take place in the rabbit, which animal shows no increase in hemoglobin concentration with epinephrine or in conditions of excitement.

11. Finally these experiments demonstrate that in dealing with hemoglobin concentration and "blood volumes" the question of red cell distribution must be taken into account.

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BIO-PHYSICAL STUDIES OF THE EFFECTS OF
VARIOUS DRUGS UPON THE TEMPERA-
TURE OF THE BRAIN AND THE
LIVER¹

I—STRYCHNIN, II—MORPHIN, III—BROMIDES,
IV—CURARE, V—ATROPIN, VI—CAFFEIN,
VII—ALCOHOL

GEORGE W. CRILE, AMY F. ROWLAND AND S. W. WALLACE

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In previous reports of our thermo-electric studies of temperature variations in animal tissues we have noted that the injection of adrenalin in normal animals was uniformly followed by a rise in temperature of about $0.5^{\circ}\text{C}.$, the periods of rise and fall being practically uniform, and the curve being completed in approximately ten minutes.² If further studies should prove that we are correct in our assumption that variations in the temperature of the brain indicate alterations in its oxidative power, then this response to adrenalin would be an indication of the oxidative power of the brain and it should be possible to compare the effects of various agents upon the oxidative power of the brain by measuring the response of the brain to the injection of adrenalin after the injection or application of those agents. In the following studies therefore the effects of various drugs upon the temperature of the brain and also of the liver have been observed, the variations in temperature being measured by means of specially constructed thermocouples.

¹ Read before the Pharmacological Society of the Federation of American Societies for Experimental Biology, Toronto, Ont., December 27-29, 1922.

² Amer. Jour. Physiol., 1922, lxii, 370-382; Proc. Amer. Phil. Soc., 1922, lxi, 237-245.

STRYCHNIN

In a previous report³ we have stated that the effects of strychnin upon the temperature of the brain vary in relation to the clinical effects. That is, in an animal in which typical convulsions occurred there were correspondingly great variations in the brain temperature while in another in which the muscular contractions were less marked the variations in the temperature of the brain were correspondingly less than in the former instance. In no instance was the temperature of the liver notably affected (fig. 1).

For the further interpretation of these findings, in view of the conclusions summarized above, 6 animals were given a standard dose of adrenalin (0.4 cc. of a 0.001 per cent solution—P. D. & Co.—per kilogram) after the injection of varying doses of strychnin. The resultant effects upon the temperature of the brain and the liver are shown in table 1. The interesting feature of these observations is the opposite effect of the injection of adrenalin in the presence of strychnin upon the temperature of the brain and of the liver. The rise in the temperature of the brain after the first dose of adrenalin varied from 0.25 to 1.2°C; the fall in the temperature of the liver varied from 0.4 to 1.8°C. The marked fall in the temperature of the liver is noteworthy in contradistinction to the lack of effect of strychnin alone upon the temperature of the liver which has been noted above (fig. 2).

MORPHIN

In view of the diversity of opinion regarding the influence of morphin on the consumption of oxygen and of the known fact that morphin acts primarily upon the higher centers in the cortical portion of the brain, it seemed of especial importance to study the effect upon the temperature of the brain of the injection of adrenalin in the presence of narcotization by morphin. Five experiments were performed, the results of which are given in table 2. A study of this table shows that the amount

³ Amer. Jour. Physiol., 1922, lxii, 349-369.

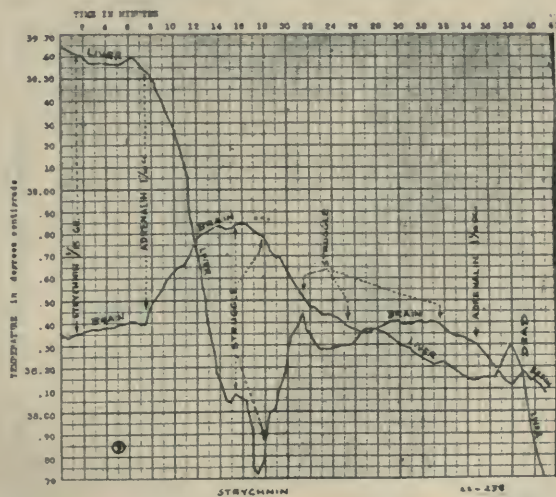
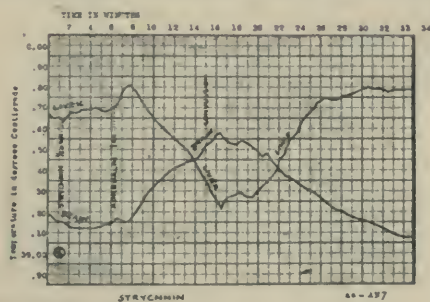
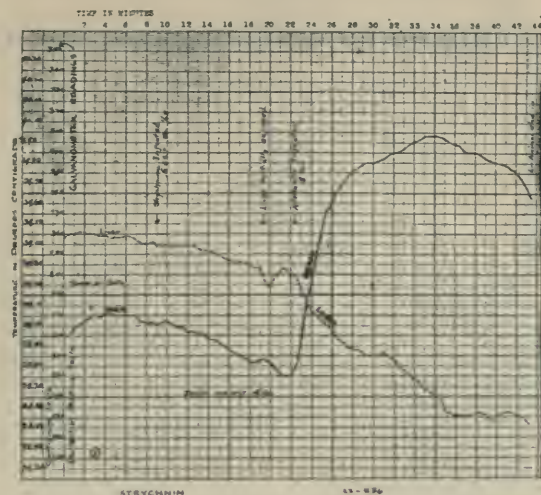


FIG. 1. EFFECT OF THE INJECTION OF ADRENALIN ON THE TEMPERATURE OF THE BRAIN AND OF THE LIVER OF ANIMALS WHICH HAD RECEIVED A PREVIOUS DOSE OF STRYCHNIN

TABLE I
The effect of strychnin upon the oxidative power of the brain and the liver as indicated by temperature changes after the injection of adrenalin

EXPERIMENT NUMBER	STRYCHNIN DOSAGE	TIME AFTER STRYCHNIN ADRENALIN WAS GIVEN		MAXIMUM RISE OF BRAIN TEMPERATURE	DURATION OF RISE IN BRAIN TEMPERATURE	MAXIMUM FALL OF LIVER TEMPERATURE	DURATION OF FALL OF LIVER TEMPERATURE
				°C.	minutes	°C.	minutes
I	$\frac{1}{60}$ grain—3 doses (intramuscular) at 10 minute intervals	Dose I	8 minutes after last dose of strychnin	+0.25	6	-0.40	9
		Dose II	21 minutes after last dose of strychnin	+0.10	2	-0.30	5
II	$\frac{1}{20}$ grain (intramuscular)	Dose I	5 minutes after last dose of strychnin	+0.6	7	-0.40	13
		Dose II	44 minutes after last dose of strychnin	+1.0*	14	-0.17	8
III	$\frac{1}{100}$ grain (intravenous)	Dose I	5 minutes after dose I of strychnin	+0.5	6	-1.15	5
		Dose II	5 minutes after dose II of strychnin	+0.6	9	-0.8	7

I

II

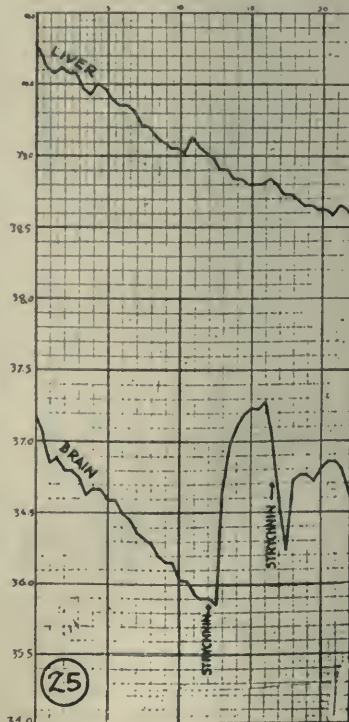
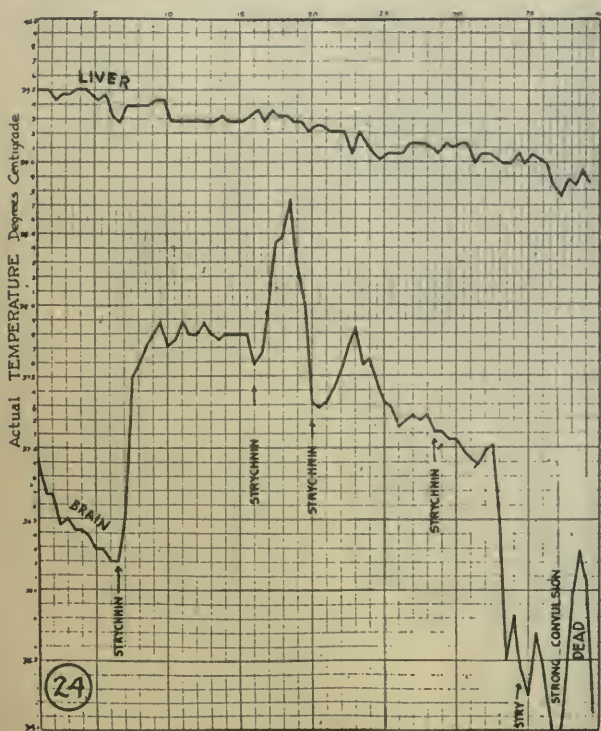
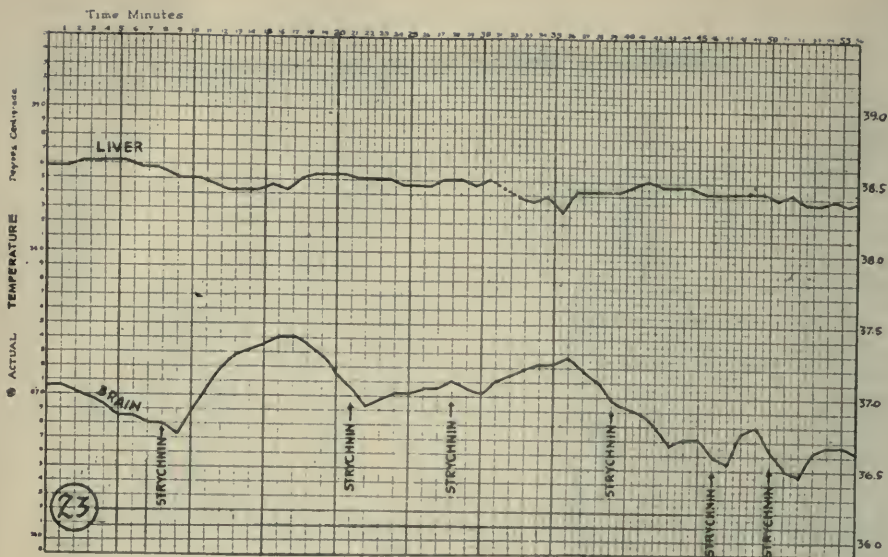
III

Convulsions

IV	Dose I	$\frac{1}{2}$ grain (intramuscular)	Dose I	5 minutes	+0.42	10	-0.5	10	Convulsions
V	Dose I	$\frac{1}{8}$ grain (intramuscular)	Dose I	6 minutes	+0.45	9	-1.8	10	Convulsions
VI	Dose I	$\frac{1}{8}$ grain (intramuscular)	Dose I	13 minutes	+1.2	14	-0.7	16	Died in convulsions

The dose of adrenalin in each experiment was 0.4 cc. of 1:1000 (P. D. & Co.) per kilogram.

* This followed marked fall in brain temperature after first dose of adrenalin.



STRYCHNIN Brain- Liver

FIG. 2. EFFECT OF THE INJECTION OF STRYCHNIN UPON THE TEMPERATURE OF THE BRAIN AND OF THE LIVER
Note the lack of response in the liver

TABLE 2
The effect of morphin upon the oxidative power of the brain and the liver as indicated by temperature changes after the injection of adrenalin

EXPERIMENT	WEIGHT OF RABBIT	DOSE OF MORPHIN SUBCUTANEOUSLY	DOSE OF ADRENALIN INTRAVENOUSLY	PERIOD BETWEEN MORPHIN INJECTION AND INJECTION OF ADRENALIN	BRAIN		LIVER		STATE OF NARCOSIS
					Total rise after adrenalin	Dura- tion of rise after adrenalin	Total fall after adrenalin	Dura- tion of fall after adrenalin	
					°C.	minutes	°C.	minutes	
I	5½	2	Dose I 1.0	38 min.	+0.2	5	0	0	Deep
	5½		Dose II 1.0	1 hr. 15 min.	+0.2	6	-0.1	6	Deep
	5½		Dose III 1.0	3 hrs. 50 min.	+0.17	6	-0.05	4	Deep
II	5½	2	Dose I 1.0	1 hr. 5 min.	+0.15	5	-0.05	4	Deep
	5½		Dose II 1.0	3 hrs. 22 min.	+0.4	8	-0.22	11	Slight (rabbit nearly recovered)
III	7	2	Dose I 1.25	1 hr. 8 min.	+0.5	10	-0.8	9	Very slight
	7		Dose II 1.25	3 hrs. 22 min.	+0.4	11	-0.65	9½	Practically none
IV	5½	2	Dose I 1.0	1 hr. 5 min.	+0.05	5	0	0	Deep
	5½		Dose II 1.0	1 hr. 38 min.	+0.2	5	0	0	Fairly deep
	5½		Dose III 1.0	2 hrs. 44 min.	+0.25	4	0	0	Partial (rabbit struggled frequently)
V	5	2	Dose I 1.0	42 min.	+0.3	6	Liver readings made		Dose I: partial (rabbit had not fully gotten effect of morphin).
			Dose II 1.0	1 hr. 20 min.	0				Dose II: very deep (pinching of femoral nerve gave practically no response)

of increase in the temperature of the brain after the injection of adrenalin was in direct relation to the depth of narcosis of the rabbit, varying from 0 and 0.05 to 0.2°C. in deeply narcotized rabbits, and from 0.25°C. in a slightly narcotized rabbit to 0.5°C. in a rabbit in which the morphin produced scarcely any clinical effects. The effect of adrenalin upon the temperature of the liver was in the opposite direction to that upon the temperature of the brain and in general this effect also was in direct relation to the depth of the narcosis. In 4 cases no response was noted in the liver temperature; but in one very strongly narcotized rabbit a very marked fall in temperature was noted after each of 2 injections of adrenalin—of 0.8 and 0.65°C., respectively (fig. 3).

BROMIDES

In 6 experiments 15 cc. of a 20 per cent solution of sodium bromide was injected intravenously on each of three consecutive days. At varying periods after the injection on the third day a standard dose of adrenalin was injected. No variation from the normal response to adrenalin was observed. There was no notable effect upon the temperature of the liver.

CURARE

It appeared that it might be of interest to establish the temperature variation in the brain in the absence of any metabolic activity in the voluntary muscular system. Since curare acts specifically on the neuro-muscular plates its injection would cut down the metabolism of the muscular system as a whole. In 2 animals therefore 10 mgm. of a 0.5 per cent solution of curare was injected intramuscularly. Artificial respiration was established and the usual injection of adrenalin was given. The only alteration in the response of the brain was that the temperature rise was somewhat delayed and was less than the normal *average* response but not less than the response observed in some normal animals. There was no effect upon the temperature of the liver.

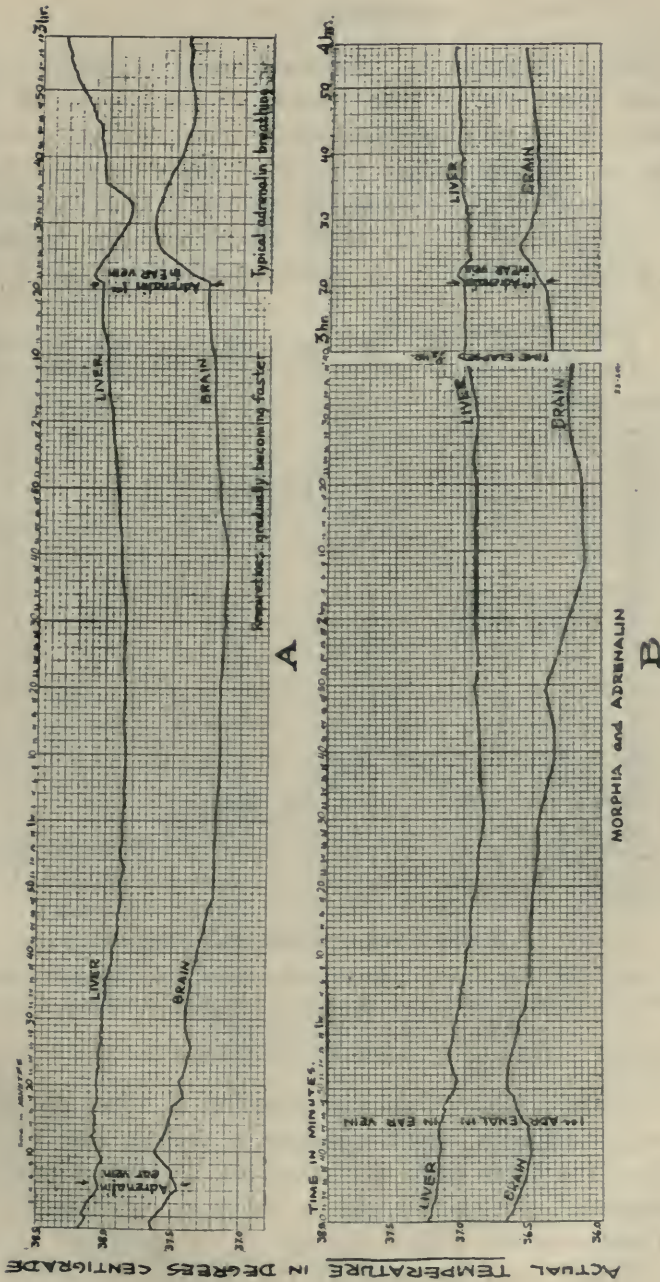


FIG. 3. EFFECT OF THE INJECTION OF ADRENALIN IN A MORPHINIZED ANIMAL

In A note the lack of response after the first injection when the animal was in deep narcosis with the almost normal response after the second injection when the animal had almost recovered from the effect of the morphin.

ATROPIN

Since atropin produces an increased blood pressure as the result of the contraction of the arteries of the splanchnic area and to some extent produces peripheral vasodilation, it would appear that in all probability the blood supply to the brain would be increased by the injection of atropin. If the increased temperature of the brain which follows the injection of adrenalin in normal animals is due to an increased blood supply to the

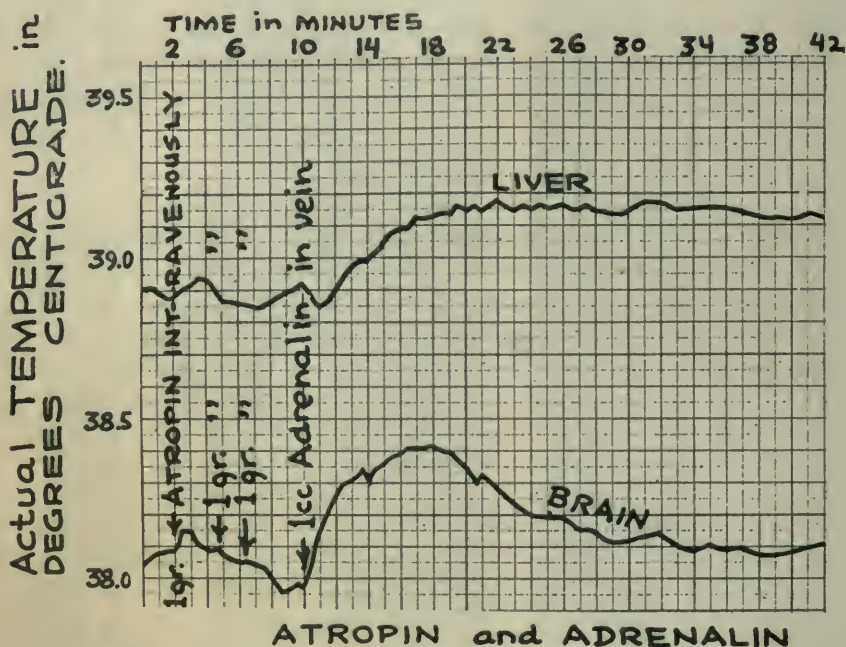


FIG. 4. EFFECT OF THE INJECTION OF ADRENALIN UPON THE TEMPERATURE OF THE BRAIN AND OF THE LIVER IN AN ANIMAL WHICH HAD RECEIVED PRECEDING DOSES OF ATROPIN

Note the increase in temperature in both brain and liver

brain then it would follow that the injection of adrenalin after the injection of atropin would produce a greater rise in the temperature of the brain than that observed in normal animals. Three experiments were performed in which no variation from the normal response was noted, the rise in temperature in the 3 rabbits utilized being respectively 0.6, 0.45 and 0.4°C. In

contradistinction to other experiments, however, a gradual rise in the temperature of the liver of between 0.3 and 0.4°C . was noted (fig. 4).

CAFFEIN

Since caffein stimulates especially the cortical centers of the brain, it was suggested that it might be of value to note its effect upon the temperature of the brain. Two experiments were performed in which each rabbit was given 2 subcutaneous doses of caffein, of 0.5 grain each ten minutes apart. The subsequent injection of adrenalin was followed by a rise in temperature which did not vary in amount from that observed in normal animals but did vary from the normal response in its abruptness. That is, the rise began while the injection of adrenalin was being given and the temperature rose to the maximum point with extreme rapidity. No notable change in the temperature of the liver was observed.

ALCOHOL

Variance of opinion as to the physiologic effect of alcohol suggested the use of this agent in this series of studies. In 1 rabbit 20 cc. of a 50 per cent solution of alcohol was introduced into the stomach and in 2, 8 cc. of a 25 per cent solution was given intravenously. In each instance the temperature of the brain fell with a rapidity corresponding to the fall in an animal in a moderate degree of shock. Periods of muscular activity occurred at almost equal intervals, each causing a momentary rise in the temperature of the brain and the liver amounting to about 0.07°C . The injection of adrenalin in these animals caused a rise in the temperature of the brain corresponding to that in normal animals with an abrupt fall after the maximum point had been reached to a point which in one instance was 1.7 degrees below the temperature when the adrenalin was given. In another case the animal died suddenly when the temperature of the brain had fallen to a point 0.35 degree below that at which the adrenalin was given (fig. 5).

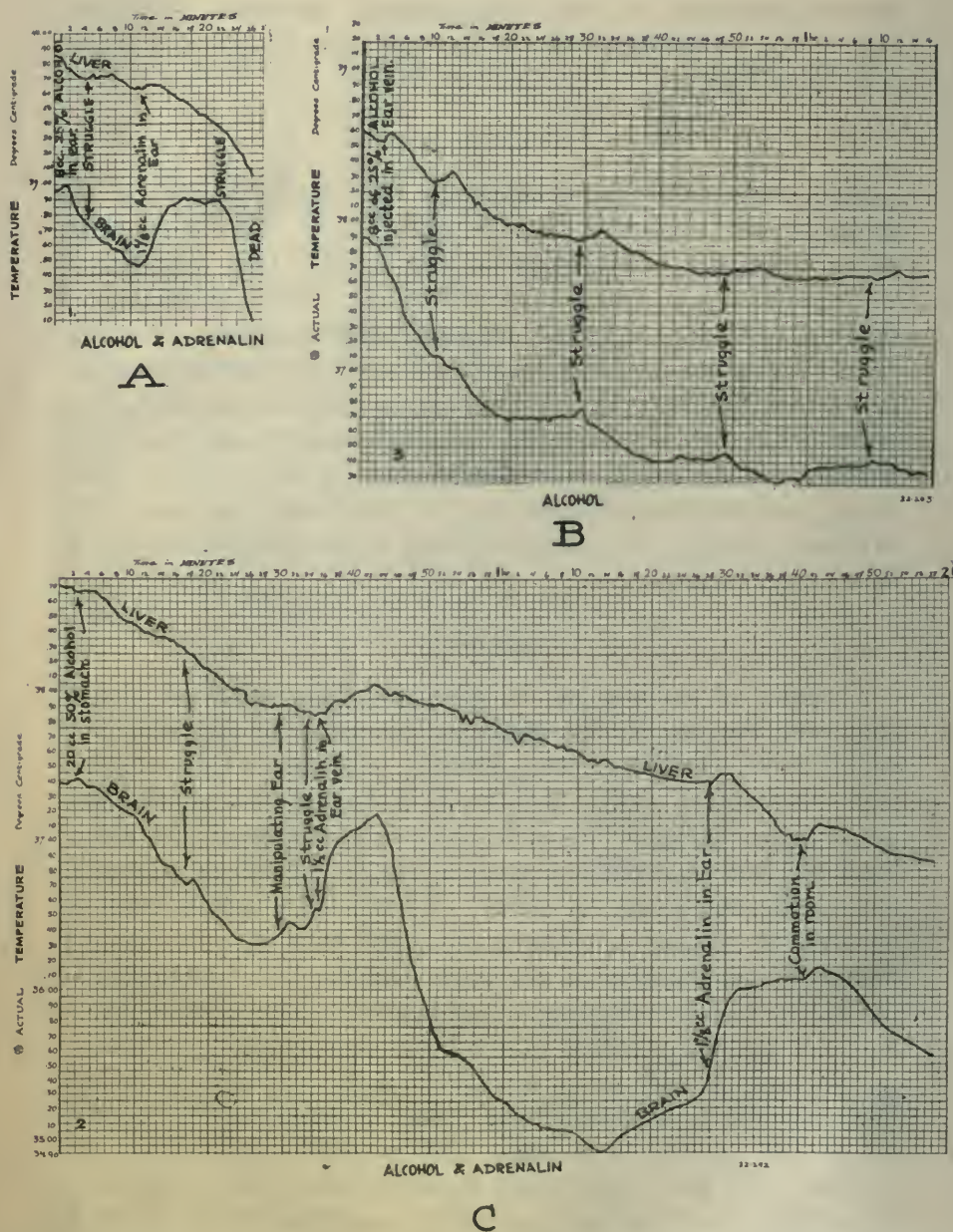


FIG. 5. THE EFFECT OF ALCOHOL UPON THE TEMPERATURE OF THE BRAIN AND OF THE LIVER, B, AND OF THE INJECTION OF ADRENALIN IN ANIMALS WHICH HAD RECEIVED PRECEDING DOSES OF ALCOHOL, A AND C

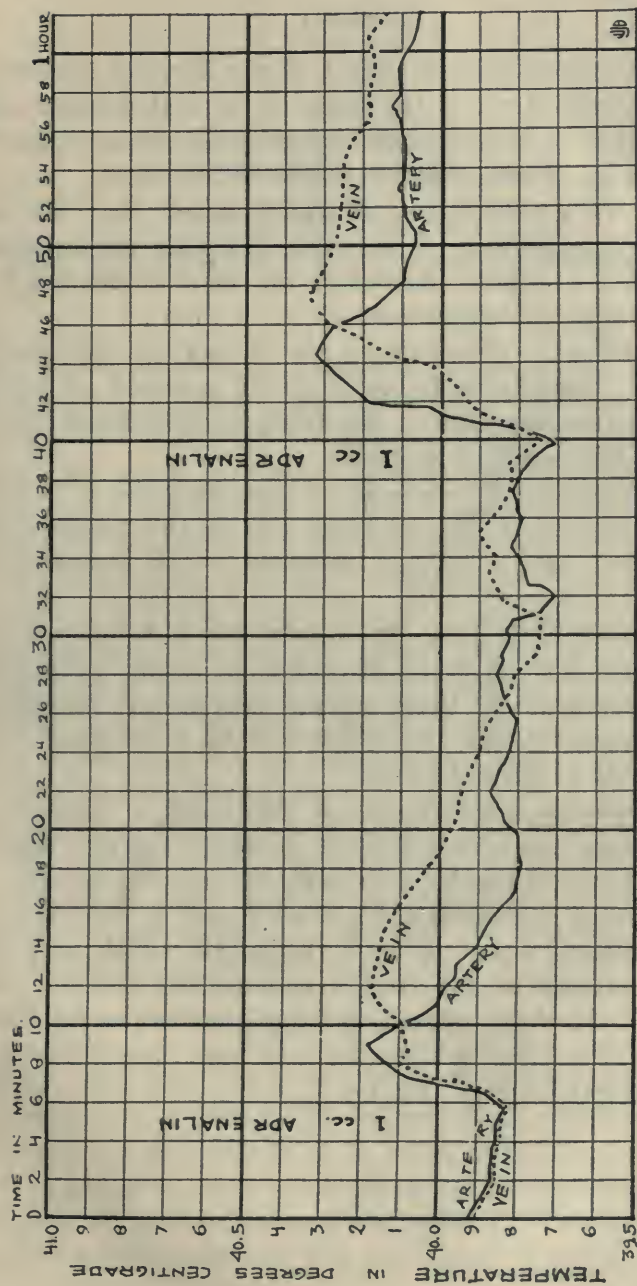


FIG. 6. THE COMPARATIVE EFFECTS OF THE INJECTION OF ADRENALIN UPON THE TEMPERATURE OF ARTERIAL (CAROTID) AND VENOUS (JUGULAR) BLOOD

SUMMARY

1. It is assumed that the effect of the injection of adrenalin upon the temperature of the brain in normal animals can be used as a unit of measurement whereby to estimate the effect of an agent upon the oxidative power of the brain.

2. From these experiments it would appear that the alteration in the temperature of the brain following the injection of adrenalin is not notably effected by the effect of the agent upon the blood supply of the brain.

3. The following observations are of especial significance:

a. When adrenalin is injected in the presence of morphin, the temperature response of the brain is diminished, in direct relation to the depth of narcosis.

b. The injection of adrenalin in the presence of strychnin produces not only a characteristic rise in the temperature of the brain but also a marked decrease in the temperature of the liver.

c. Alcohol of itself alone produces a fall in temperature corresponding to that observed in rabbits in shock; while the injection of adrenalin in these animals produces a characteristic rise in temperature; this rise is followed by a fall which exceeds that observed in normal rabbits.

Note: Since the presentation of this paper a further series of experiments has been initiated, in which thermocouples are placed within the jugular vein and the carotid artery. The first experiment is illustrated by chart 6 and is cited here only as a preliminary report of an investigation which may aid in the interpretation of the studies summarized above. As will be noted by the chart, after each injection of adrenalin the temperature of the blood within the vein rose above that of the blood within the artery (fig. 6).

A PHARMACOLOGICAL AND CLINICAL EXAMINATION OF BENZYL MANDELATE

DAVID I. MACHT

From the Pharmacological Laboratory, the Johns Hopkins University

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In a study of the pharmacological properties of benzaldehyde (1) the author has shown that this compound exhibits the same antispasmodic properties for smooth muscle as were noted by him in the case of the benzyl esters, benzyl benzoate, benzyl acetate, benzyl succinate and benzyl nitrite (2); as also in the case of benzyl alcohol (3). Furthermore the author pointed out that mandelic acid, a simple derivative of benzaldehyde, also possesses to a certain degree these antispasmodic properties (4). These properties were exhibited mildly by the simple salt, sodium mandelate and very much more markedly by various esters of mandelic acid such as ethyl mandelate, etc. It occurred to the author that if a new ester, benzyl mandelate, were synthesized, it might possibly exhibit such antispasmodic properties to an even greater degree inasmuch as both the alkyl and the acid radicals of such a compound possess the same pharmacological characteristics. Accordingly at the suggestion of the author such an ester was synthesized for him by Dr. Daniel Base and its pharmacological properties were studied.

CHEMICAL PROPERTIES

Benzyl mandelate is a new ester of mandelic acid never having been synthesized before. This is a beautiful, colorless crystalline body. Its melting point is 93°C. It is very little soluble in cold water and slightly soluble in boiling water but is freely soluble in alcohol, ether, benzene and chloroform. Solutions in alcohol are readily precipitated by the addition of water to make 40 or

50 per cent alcohol. In boiling water less than 1 mgm. will dissolve in 10 cc. The method of preparation of this compound is described by Base elsewhere (5).

TOXICITY

In view of the low toxicity of such benzyl esters as the benzoate, succinate, etc., on the one hand and of the very low toxicity of mandelic acid, it was found as might be expected that benzyl mandelate is not very poisonous given by stomach tube to rabbits. Doses of 0.5 ^{g.m.}mgm. were certainly without deleterious immediate or late effects. The same was true for dogs. The exact lethal dose by mouth for these animals could not be determined for lack of sufficient material. Even when injected intravenously into dogs in the form of emulsions benzyl mandelate produced no alarming symptoms or after effects. Doses of 100 mgm. at a time were given repeatedly to dogs in this way. Intravenous injections in rabbits of emulsions of benzyl mandelate, containing 50 mgm. in 10 cc. were also not fatal. Injections of benzyl mandelate, 200 mgm. dissolved in oil and administered intraperitoneally in rabbits produced no dangerous symptoms. The author himself took by mouth capsules of benzyl mandelate containing as high as 250 mgm. without experiencing any discomfort or disagreeable symptoms.

PHARMACOLOGY

A pharmacological examination of benzyl mandelate was made especially in regard to possible local anesthetic action and the characteristic antispasmodic effects on smooth muscle shown by other benzyl esters. Observations were also made on the blood pressure and the effects on the heart and respiration. Owing to the very poor solubility of the ester in water considerable difficulty was encountered in experimenting with it especially on whole animals. The drug however, was found to be sufficiently soluble in warm water to produce definite pharmacological effects on isolated tissues and organs. For intravenous and intraperitoneal injections saturated solutions of benzyl mande-

late in warm saline were used in some cases while in others when it was desired to introduce larger doses of the drug, it was necessary to resort to the use of emulsions.

Anesthetic effect. Owing to the very poor solubility of benzyl mandelate no effect on the sensory nerve endings could be demonstrated.

Effect on smooth muscle. As was to be expected the effect of even very weak solutions on benzyl mandelate on isolated smooth muscle preparations was to produce a marked relaxation of the

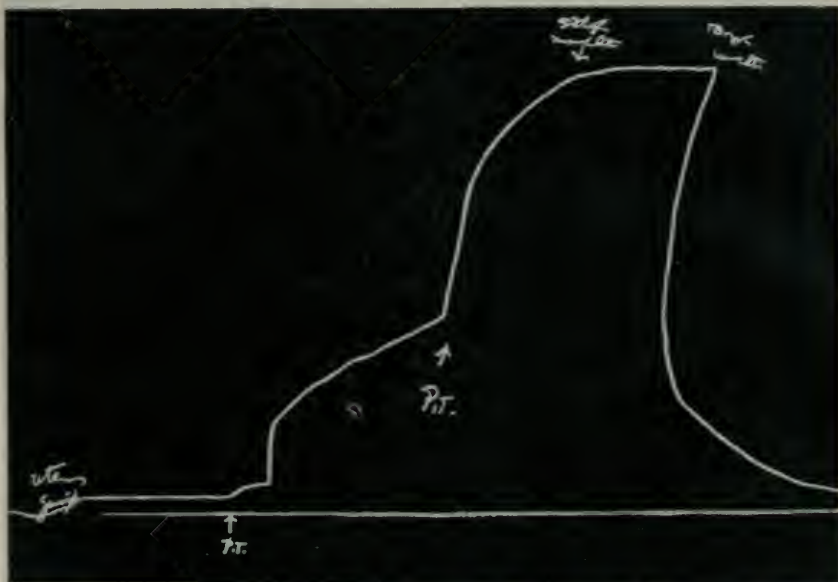


FIG. 1. UTERUS OF A GUINEA-PIG, STIMULATION WITH PITUITARY LIQUID AND RELAXATION WITH BENZYL MANDELATE

same. Experiments were made on excised preparations from the uterus, intestine, urinary bladder, gall bladder and other organs. The results in every case being the same, as illustrated by the accompanying figures.

Figure 1 shows the effect of an aqueous solution of benzyl mandelate on the uterus of a virgin guinea-pig. The uterus was first brought into a state of powerful contraction by two doses

of 0.1 cc. each of pituitrin. At the height of contraction a solution containing four milligrams of ethyl mandelate was introduced but was found ineffective in counteracting the powerful contractions produced by the pituitary extract. On addition of 5c c. of a saturated solution of benzyl mandelate, however, a complete relaxation of the uterus rapidly followed (fig. 1).

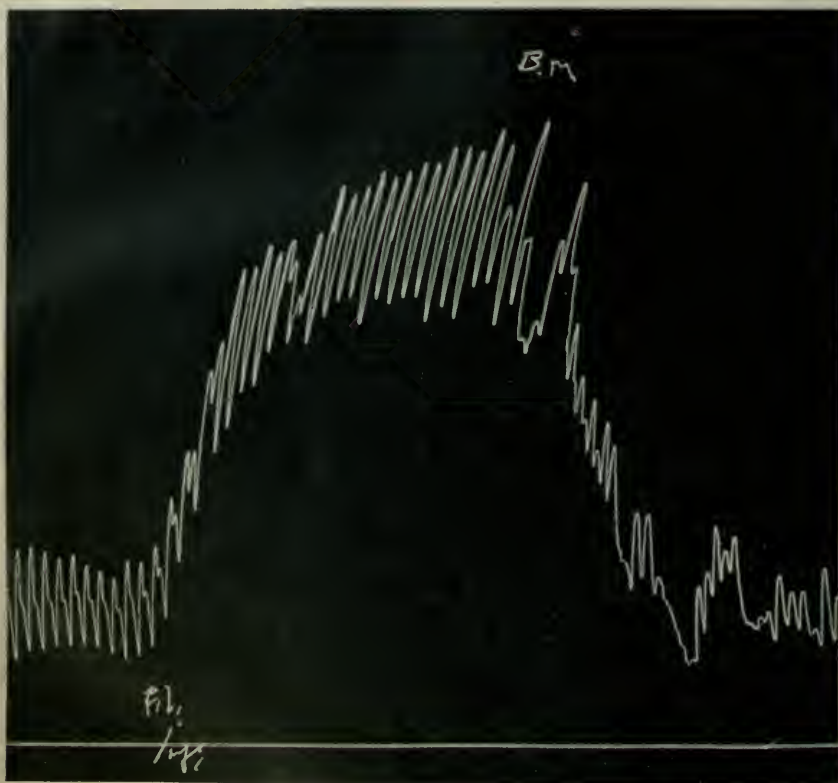


FIG. 2. SMALL INTESTINE OF RABBIT, STIMULATION WITH PILOCARPIN HYDROCHLORIDE, RELAXATION WITH BENZYL MANDELATE

Figure 2 illustrates the effect of benzyl mandelate on an isolated preparation of a rabbit's jejunum. This was first stimulated to contraction by the introduction of 1 mgm. of pilocarpin hydrochloride in 50 cc. of Locke's solution and then relaxed by the addition of 3 cc. of a saturated solution of benzyl mandelate (fig. 2).

Figure 3 illustrates the effect of benzyl mandelate solution on the small intestine of a rat. In this case the preparation was brought into a tonic contraction with 2 mgm. of pilocarpin hydrochloride in 40 cc. of Locke's solution and relaxed with 5 cc. of a saturated solution of benzyl mandelate in water (fig. 3).



FIG. 3. SMALL INTESTINE OF RAT, STIMULATION WITH Pilocarpin Hydrochloride, Relaxation with Benzyl Mandelate

The author was fortunate in securing several surviving preparations of human organs from various surgical operating rooms, and in this way he duplicated the results obtained in lower animals in the case of human beings. Figure 4 illustrates the effect of

benzyl mandelate on a bit of muscle obtained from a human uterus after a case of complete hysterectomy. The preparation was brought into a state of contraction by means of 2 mgm. of ergotoxin phosphate in 40 cc. of Locke's solution and was subsequently relaxed by the introduction of 5 cc. of a saturated solution of benzyl mandelate in water (fig. 4). Figure 5 shows

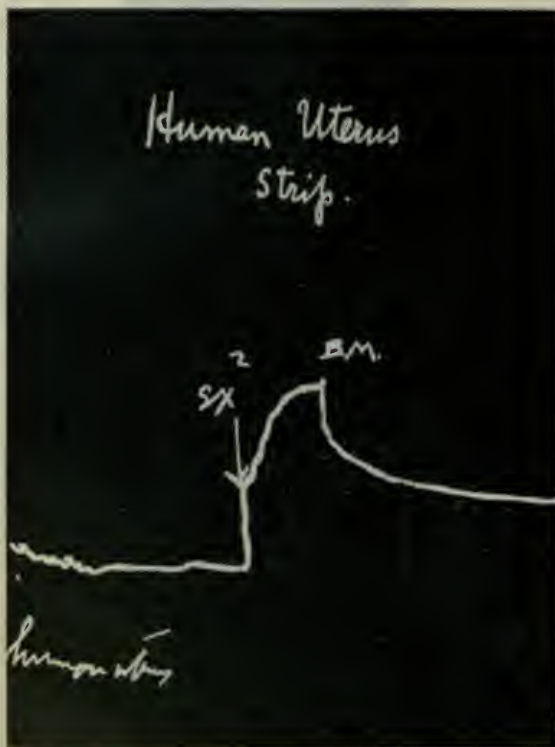


FIG. 4. MUSCLE STRIP FROM HUMAN UTERUS, CONTRACTED BY ERGOTOXIN AND RELAXED BY BENZYL MANDELATE

the effect of various drugs on the excised muscle from a human gall bladder. At *m*, 5 mgm. of morphine sulphate were introduced into the chamber containing 40 cc. Locke's solution and produced a slight contraction. This was followed by 1 mgm. of pilocarpin hydrochloride which produced a much greater contraction of the gall bladder muscle. The spasm was then relaxed by the introduction of 5 cc. of a saturated solution of benzyl mandelate (*b.m.*) (fig. 5).

Blood pressure. Experiments on blood pressure were made on dogs, cats and rabbits. Great difficulty was encountered in the attempts to introduce benzyl mandelate intravenously. The use of saturated *aqueous* solutions in dogs and cats was followed by very little effect. In rabbits however, such injections were followed by a marked fall in blood pressure. This agreed with



FIG. 5. MUSCLE STRIP FROM HUMAN GALL BLADDER, STIMULATION BY MORPHINE, MARKED CONTRACTION BY PILOCARPIN, RELAXATION BY BENZYL MANDELATE

the experiences of the authors with benzyl benzoate, published elsewhere, where the author called attention to the greater efficiency of the drug for herbivora. Figure 6 illustrates the effect of the injection of a saturated solution of benzyl mandelate into the vein of a rabbit. It will be noted that the injection of

2 cc. was followed by a marked fall. It was found by the author that injections of emulsions of suspensions of benzyl mandelate intraperitoneally were also followed by a fall in blood pressure, which indeed was of a longer duration than after intravenous

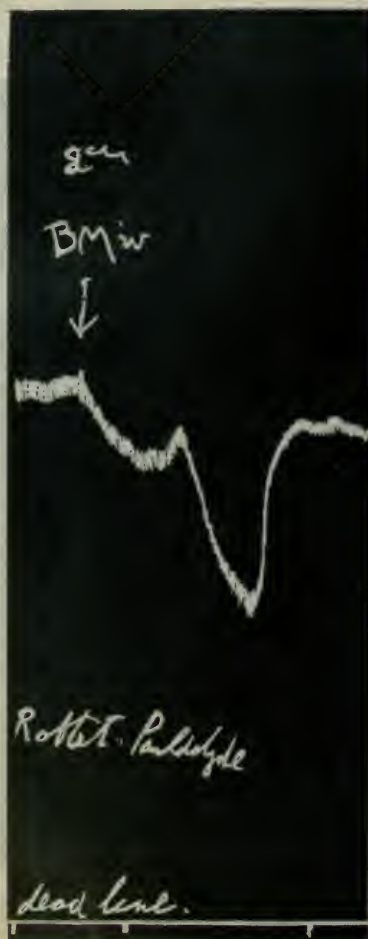


FIG. 6. BLOOD PRESSURE CURVE IN THE RABBIT UNDER PARALDEHYDE ANESTHESIA, SHOWING THE EFFECT OF BENZYL MANDELATE INJECTED INTRAVENOUSLY

injections. Again, the introduction of benzyl mandelate into the stomach through a stomach tube was also followed by a slow but prolonged fall in blood pressure as illustrated in figure 7.

The results obtained by intraperitoneal and oral injections of the drug agree well with the experience of the author as well as other

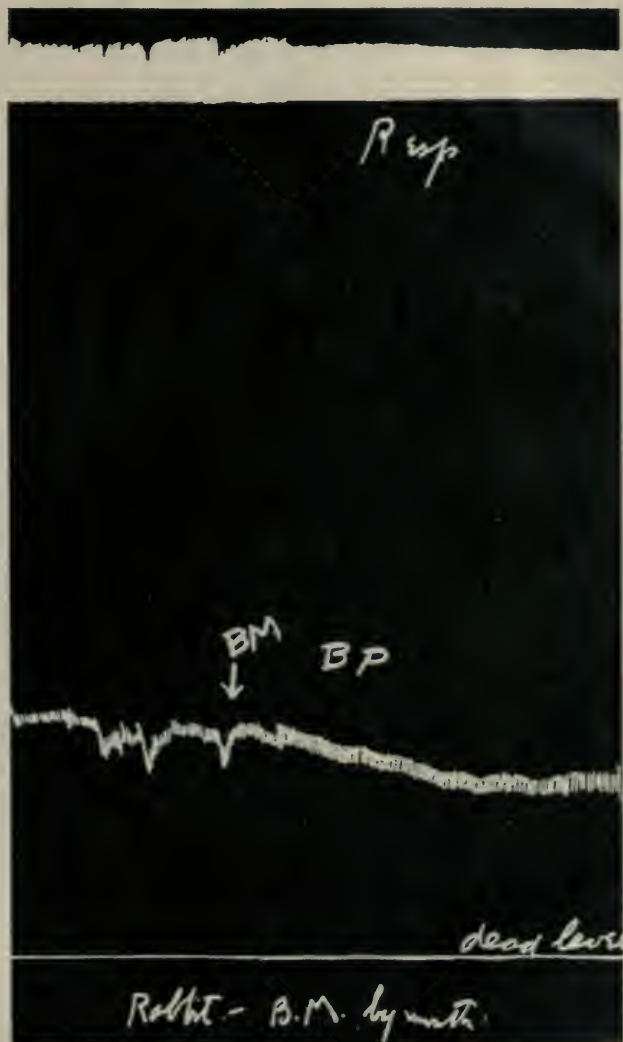


FIG. 7. RABBIT UNDER PARALDEHYDE ANESTHESIA. EFFECT ON RESPIRATION AND BLOOD PRESSURE OF BENZYL MANDELATE INTRODUCED INTO THE STOMACH

writers (7) with various benzyl esters in which it was found that the pharmacological or therapeutic efficiency of these esters runs

Table 1, pp. 452-453, instead of grains should read grams.

TABLE 1

SUBJECT	SYSTOLIC DIASTOLIC PRESSURES BEFORE DRUG	DRUG	SYSTOLIC DIASTOLIC PRESSURES AFTER DRUG	TIME OF ACTION
1. Hoff.....	230 145 min.	Benzyl mandelate 0.1 grain	190 125 min.	45 minutes
2. Jones.....	210 130 min.	Benzyl mandelate 0.1 grain	195 120 min.	45 minutes
3. Schaeffer.....	220 130 min.	Benzyl mandelate 0.1 grain	185 115 min.	45 minutes
4. Hamilton.....	205 130 min.	Benzyl mandelate 0.1 grain	190 120 min.	45 minutes
5. Ge. B.....	192 138 min.	Benzyl mandelate 0.06 grain	180 120 min.	45 minutes
6. Scott.....	225 110 min.	Benzyl mandelate 3 doses, 0.05 grain	180 100 min.	12 hours
7. Macleod.....	260 140 min.	Benzyl mandelate 0.1 grain	238 124 min.	45 minutes
8. E. M.....	220 130 min.	Benzyl mandelate 0.1 grain	200 110 min.	1 hour
9. Brooks.....	200 96 min.	Benzyl mandelate 0.1 grain	185 94 min.	45 minutes
10. Emb.....	230 135 min.	Benzyl mandelate 0.1 grain	215 120 min.	1 hour
11. Norris.....	174 110 min.	Benzyl mandelate 0.1 grain	162 100 min.	45 minutes

12. C. D.....	$\frac{136}{100}$ min.	Benzyl mandelate 0.1 grain <i>8 min</i>	$\frac{126}{95}$ min.	30 minutes
13. C. D. R.....	$\frac{136}{100}$ min.	Benzyl mandelate 0.1 grain <i>8 min</i>	$\frac{125}{94}$ min.	1 hour
14. Jo. S.....	$\frac{200}{130}$ min.	Benzyl mandelate 0.1 grain <i>5 min</i>	$\frac{190}{115}$ min.	45 minutes
15. Robb.....	$\frac{215}{100}$ min.	Mandelic acid 0.25 grain <i>5 min</i>	$\frac{195}{90}$ min.	1 hour
16. Corp.....	$\frac{205}{130}$ min.	Mandelic acid 0.2 grain <i>5 min</i>	$\frac{190}{120}$ min.	40 minutes

more or less parallel to the degree and rapidity of their hydrolysis or dissociation. Such a break up of the esters probably takes place just as rapidly or even more rapidly when given by mouth than when injected intravenously (figs. 6 and 7).

Effects on the heart and respiration. Even after injections of large amounts of benzyl mandelate in emulsions by the intravenous route very little depression of the heart or respiration was noted. Inasmuch as 50 mgm. of the drug in the form of an emulsion were injected intravenously in rabbits without any very toxic effects, the author has found this compound to be certainly not more toxic in this respect than the other esters studied by him such as benzyl benzoate and benzyl succinate.

CLINICAL EXPERIENCES

In view of the low toxicity of benzyl mandelate and in view of the wide therapeutic employment of other benzyl esters in medical practice it was deemed quite safe to administer benzyl mandelate in small doses to a number of normal healthy individuals and subsequently also to a number of patients exhibiting hypertension or high blood pressure. The results were gratifying. The subjoined table gives the systolic and diastolic blood pressures of a number of cases studied by the author before and after administration of benzyl mandelate. The drug in each case was administered by mouth either in powdered form or in capsules and the blood pressure was measured one-half hour to an hour after the administration of the drug. The blood pressures were determined carefully by the auscultatory method with a sphygmomanometer of the Tycos type and whenever possible the readings were checked up by more than one observer. In a few cases graphic records of the blood pressure before and after the administration of the drugs were made with the Erlanger apparatus. It was found in every case that the administration of even small doses of benzyl mandelate (1 to 2 grains) was followed by a definite systolic and diastolic fall in blood pressure. In no case were there any untoward symptoms observed by the physician or complained of by the subject. On the other hand in a number of cases the patients stated that their symptoms were

distinctly relieved. The author has also made a few observations on the effect of benzyl mandelate in other clinical cases, such as those of dysmenorrhea, asthma and diarrhea, but the data so far in hand are too few to warrant any definite statement on the subject.

Inasmuch as the author has shown that mandelic acid itself is also a relaxant of smooth muscle a number of clinical observations were made with that drug. Administration of small quantities (2 to 5 grains), of mandelic acid in solution by mouth to patients were also followed by a distinct fall in blood pressure within thirty minutes. The effects of mandelic acid alone, however, were not as potent as those of the benzyl ester.

SUMMARY

A pharmacological and toxicological study of the new compound benzyl mandelate was made. It was found that this compound is of comparatively low toxicity on the one hand and is pharmacologically effective on the other hand. The most important pharmacological action of the drug is on smooth muscle. Benzyl mandelate produces a marked relaxation of all such preparations especially after they have been brought into a state of tonic or marked contraction. In view of the antispasmodic pharmacological properties of both the alkyl and acid components of the molecule a greater antispasmodic effect is produced by the compound than by other benzyl esters. This fact taken together with the lack of disagreeable taste of benzyl mandelate gives promise of its having considerable therapeutic interest.

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